**Universidade de Aveiro**
**Ano 2013**
Departamento de Biologia

**Ana Catarina Neves Ferreira Lino**

# Dieta e ocupação de um abrigo de criação de *Rhinolophus hipposideros*

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Aplicada – Ramo de Ecologia, Biodiversidade e Gestão de Ecossistemas, realizada sob a orientação científica do Prof. Dr. Carlos Manuel Martins Santos Fonseca, Professor Auxiliar com Agregação do Departamento de Biologia da Universidade de Aveiro e coorientação científica da Doutora Maria João Ramos Pereira, Investigadora de pós-doutoramento do Centro de Estudos do Ambiente e do Mar da Universidade de Aveiro.

À minha querida Ceninha…
A toda a minha família…

**o júri**

presidente

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**palavras-chave**

abrigo de maternidade, dieta, fatores abioticos, floresta Mediterrânica, nascimentos, *Rhinolophus hipposideros*, seleção de presas.

**resumo**

*Rhinolophus hipposideros* (Bechstein, 1800) sofreu um severo declínio ao longo da sua área de distribuição estando já extinto em alguns países europeus. Apesar de não se conhecer com exatidão a tendência populacional desta espécie em Portugal devido à sua ampla dispersão, pensa-se que as populações estão em declínio devido principalmente à perda de abrigos adequados e à perda da qualidade de áreas de caça. Em Sintra, numa infraestrutura da Quinta da Regaleira, encontra-se uma das maiores colónias de maternidade conhecidas do país, objeto de estudo deste trabalho. O ambiente em redor deste abrigo é composto por uma floresta tipicamente mediterrânica intercalada com plantações de *Pinus pinaster*, algumas espécies exóticas e campos agrícolas.

A ocupação e a atividade dentro do abrigo e a época em que ocorrem os nascimentos foram avaliados através da análise de gravações de vídeo obtidas no interior do abrigo durante 2010 e 2012. A dieta desta colónia foi avaliada através da análise de dejetos recolhidos mensalmente e a disponibilidade alimentar foi avaliada através da amostragem de artrópodes feita com uma armadilha luminosa colocada nas proximidades do abrigo. O pico de ocupação do abrigo ocorre durante o verão, quando as fêmeas se juntam para darem à luz. Nos meses de estudo foi possível observar um padrão de atividade noturna bimodal que parece ser determinado pelos picos de intensidade de luz ao anoitecer e amanhecer. Os nascimentos ocorreram no final de maio e início de junho em 2010 e 2012, respetivamente. A dieta desta colónia é composta essencialmente por Lepidoptera, Tipulidae (Diptera), Hymenoptera, Diptera e Neuroptera. A diversidade de artrópodes manteve-se relativamente constante na dieta mas variou em termos de disponibilidade. Os resultados referentes à dieta demonstraram que *R. hipposideros* em Sintra é uma espécie generalista mas não oportunista, selecionando ativamente pequenos dípteros.

**keywords** abiotic factors, births, diet, Mediterranean forest, prey selection, *Rhinolophus hipposideros*, roost use, maternity roost.

**abstract**

*Rhinolophus hipposideros* (Bechstein, 1800) has suffered a severe decline throughout its distribution area, and is extinct in some European regions. The exact population trend in Portugal is unknown, mainly due to its wide distribution, but its populations are probably declining due to loss of suitable roosts and foraging habitats. In Sintra, an infrastructure of the "Quinta da Regaleira" harbours one of the largest known Portuguese maternity colony of this species, focus of this research. The surrounding area is mostly composed by Mediterranean woodland intercalated with *Pinus pinaster* plantations, some exotic species, and agricultural fields. Roost occupancy, activity inside the roost and time of births were evaluated through the analysis of video recordings obtained inside the roost in 2010 and 2012. Diet composition was evaluated through the analysis of faecal pellets collected monthly underneath the colony, and arthropod availability was sampled using a light trap set outside the roost. The peak of occupation occurs during summer, when females gather to give birth. There seems to be a bimodal pattern of nocturnal activity determined by the peaks of light intensity at sunset and sunrise. The highest number of flights inside of roost was registered immediately before and after the foraging periods. Births occurred in late May and early June in 2010 and 2012, respectively. The diet of this colony is essentially composed by Lepidoptera, Tipulidae (Diptera), Hymenoptera, Diptera and Neuroptera. Arthropod diversity remained fairly constant in the diet, but prey availability clearly varied along the year. *R. hipposideros* seems to be a generalist, but not an opportunistic species, actively selecting small Diptera.

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# Chapter 1

General Introduction

# 1. General Introduction

## 1.1. The systematic of Chiroptera

Around 20% of all mammal species are bats, comprising about 1300 recognized species (Wilson, 2005). Bats are unique among mammals as they have the ability of manoeuvrable flight.

Traditionally the order Chiroptera has been subdivided into two sub-orders (Simmons, 2000): i) the Megachiroptera comprising only the Pteropodidae, the flying-foxes, that feed on fruit, nectar and/or pollen distributed in tropical and sub-tropical areas of the Old World, and that lack – with the exception of a few species – the ability to echolocate, and ii) the Microchiroptera, the echolocating bats, comprising around 1000 species, and occupying all terrestrial habitats and climatic zones, with the exception of the Antarctic and some isolated oceanic islands (Hutson, 2001; Schnitzler and Kalko, 2001); microbats are the mammalian group with the largest range of dietary guilds including insectivores, carnivores, piscivores, frugivores, nectarivores and sanguinivores (Patterson et al., 2003), even if about 75% are insect hunters (Hutson, 2001).

## 1.2. Microchiropteran bats

a) Roost ecology

Bats are extremely dependent of roosts because they pass most of their life there. Microchiropteran bats select a wide range of sites for roosting both in natural and manmade structures (Hutson, 2001; Kunz, 1982; Kunz and Lumsden, 2003). Natural roost

include some ephemeral roosts as foliage, e.g. *Lasiurus cinereus* (Klug and Barclay, 2013), bird nests, e.g. *Kerivoula papuensis* (Schulz, 2000) and termite and ant nests, e.g. *Lophostoma silvicolum* (Kalko et al., 1999; Kalko et al., 2006) and *Balionycteris maculata* (Hodgkison et al., 2003). Because these roosts present unstable conditions, providing minimal protection to adverse weather, or at least only during a limited period of time, bats that use them usually show low roost-site fidelity.

Other natural roosts, but with a more permanent character, are tree holes, rock crevices and cavities. Tree holes are used in both tropical and temperate regions (Kunz and Lumsden, 2003), and provide protection against predators, adverse weather and fluctuations of ambient parameters, such as temperature and humidity (Kunz, 1982). Rock crevices have the advantage to be abundant and ubiquitous although their thermal conditions are relatively unstable. Little information is available concerning these roosts because they are difficult to find or inaccessible (Kunz, 1982). Caves and similar underground roosts, such as mines and cellars, are widely used by bats: they provide relatively constant and suitable ambient conditions for important phases of the life-cycle, including breeding and hibernation. Caves are used by many solitary bats but also by colonial bats that may constitute agglomerations of hundreds or thousands of individuals. Intraspecific competition for food and the increased incidence of parasites and diseases are some of the problems associated to these agglomerations (Kunz, 1982) these colonial habits also makes them especially vulnerable with the closure of the roosts once many species may be lost (Hutson, 2001). However, these agglomeration also have benefits namely at the level of thermoregulation (McCracken, 1989).

Usually a bat species presents some preference for some type of roosts, but the same species can occupy different roost types according to the geographic location, phase of the life-cycle and/or season (Agosta, 2002).

In the last decades, an increasing use of manmade structures as bat roosts has been found. The cause for this is probably the loss of adequate natural roosts caused by intentional or unintentional destruction, landslides and obstruction of the entries by branches or rocks. Several manmade structures, such as country houses, churches, lofts or castles, provide appropriate conditions for bats because they contain large areas and entrances that, on one hand promote the existence of different microclimates inside the roost and, on the other hand, facilitate uninterrupted flights when bats are leaving and entering the roost.

b) Diet of microchiropteran bats

The majority of microbats feeds mostly or exclusively on arthropods. However, other bats, especially those of the Phyllostomidae, developed other feeding habits: some are carnivore and feed on small vertebrates, including birds (Ibáñez et al., 2001), smaller bats and others feed on nectar, pollen and fruits, while blood is the main dietary item of three bat species, *Desmodus rotundus*, *Diphylla ecaudata* and *Diaemus youngi* (Simmons et al., 1979). The two species of Noctilionidae, *Noctilio leporinus* and *Noctilio albiventris*, are specialized in capturing and eating fish, though some bats of other families may occasionally feed on fish, such as the vespertilionids *Myotis daubentonii* (Siemers et al., 2001), *Myotis capaccinii* (Aihartza et al., 2003) and *Myotis ricketti* (Ma et al., 2003).

As already mentioned above, all microbats have the ability to echolocate. Most locate their prey through their echolocation system although some bats detect their prey using "passive listening", benefiting from the sound produced by their prey.

Bats that feed on other animals may catch their prey on the wing by aerial hawking, from the substrate such as the ground, leaves or tree bark by gleaning, and others use a vantage point from which they make short flights to catch the detected prey (Schofield, 1996).

For a review on diet, feeding strategies and prey selection by bats see Jones and Rydell (2003) and Neuweiler (2000).

## 1.3. The genus *Rhinolophus*

The Microchiroptera family of the Rhinolophidae encompasses only one genus, *Rhinolophus*, that includes 77 species (Kunz et al., 2011) and occurs in temperate, subtropical and tropical regions of the Old World.

In Europe, there are only five species of *Rhinolophus* of which four are present in Portugal. *Rhinolophus ferrumequinum* and *Rhinolophus hipposideros* that are classified as

"vulnerable" and *Rhinolophus euryale* and *Rhinolophus mehelyi* that are classified as "critically endangered" according to the Portuguese Red Data Book of Vertebrates (Cabral et al., 2005).

Molecular studies produced controversy about the interfamilial relationships of the Chiroptera, suggesting that the microbat superfamily Rhinolophoidea is more closely related to the Pteropodidae (Megachiroptera) than to any other Microchiroptera (Springer et al., 2001; Teeling et al., 2000). Despite the controversy, the traditional separation is still used.

Rhinolophids are commonly called horseshoe bats because they have a complex and conspicuous facial appendage (MacDonald and Barret, 1993; Palmeirim, 1990), composed by a nose-leaf above the nostrils and a flap of skin shaped like a horseshoe. The sella is a median projection found between the horseshoe and the nose-leaf; this structure helps to distinguish the Rhinolophidae from the Hipposideridae because these latter do not possess this structure. The nose-leaf is frequently used as a diagnostic feature as it presents slightly different shapes among species (Schofield, 1996).

The facial appendages are used to improve the targeting of the echolocation calls and are complemented by the large ears lacking a tragus (Feldhamer et al., 2007) typical of this group, specialized in receiving the echo of high frequency echolocation calls that they emit.

All species of this genus are insectivorous and specialized for flycatching and in some cases for gleaning (Norberg and Rayner, 1987) – their wings are round and relatively short, providing a very slow and highly manoeuvrable flight, and allowing bats to hunt in cluttered areas with high efficiency (Billington and Rawlinson, 2006; Norberg and Rayner, 1987). Their highly specialized echolocation system allows them to detect prey even when these are hidden in the vegetation, provided that they are flying or flapping their wings (Ma et al., 2008). Indeed, the echolocation calls of *Rhinolophus* have a special characteristic, being composed by pure tone signals (Jones and Teeling, 2006; Neuweiler, 1989); these signals maintain the call structure unaltered, even when the foliage flutters due to wind, but when a fluttering target appears, the pure tone echo will carry distinct glints creating "echo signatures" (Neuweiler, 1989; Von der Emde and Menne, 1989). Each wing beat of the target is distinctly coded by the bats, allowing them not only to detect the prey but also to identify the specific wing beatings through the analysis of the spectrum of their echoes

(Neuweiler, 1989). All echolocating bats experience a Doppler-shift in the frequency of the calls they emit compared to the frequency of the echoes they receive. However, horseshoe bats change the frequency of their calls to keep the returning echoes within a narrow frequency range where they have maximum sensitive hearing. This Doppler-shift compensation was discovered by Schnitzler (1968).

In summary, the echolocation system of horseshoe bats is the most adequate for the search of moving prey in clutter habitats and is extremely efficient in the classification of their targets (Jones and Teeling, 2006).

### 1.4. *Rhinolophus hipposideros*

The lesser horseshoe bat, *R. hipposideros* is the smallest European horseshoe bat with only 5 to 9 g, 37 to 45 mm of head-body length and 192 to 254 mm of wingspan (MacDonald and Barret, 1993). Like other rhinolophids it possesses a horseshoe-shaped nose and its ears do not have a tragus. These bats are usually brown on the back and whitish-grey on the ventral side.

When sleeping or hibernating, *R. hipposideros* completely encloses the body in the wing membranes (Norberg and Rayner, 1987).

*R. hipposideros* feeds essentially within the clutter of woodlands (Bontadina et al., 2002) and, with a sophisticated system of echolocation characteristic of rhinolophids, is capable to differentiate the characteristics of returning echoes produce by foliage and by prey. When foraging, *R. hipposideros* seems to be able only to detect prey at short distances (Schofield, 1996). In fact, it feeds on very small items. Gould (1955) estimated that smaller insects eaten by bats have equal dimensions to the wavelength of the sound emitted by these bats. The capacity of detection preys decreases sharply when the wavelength exceeds the wing length of the insect prey. Because wavelength is negatively correlated with frequency, bats with higher call frequencies are able to detect smaller insects (Jones and Rydell, 2003). Indeed, the calls of *R. hipposideros*, have short wavelength – approximately

2.94 mm – determining the size of the smallest prey item eaten (McAney and Fairley, 1989).

### 1.5. The conservation status of *R. hipposideros* in Europe and Portugal

*R. hipposideros* has a wide distribution and can be found in all Mediterranean countries (Fig. 1.1), from the western part of Europe to central Asia and from central Europe down to the northern part of Africa. Despite the wide distribution of this species, it is relatively rare in some countries e.g. Czech Republic (MoE, 2010a), Albania (MoEFWA, 2010), Moldova (MoE, 2010c) and in UK (Defra, 2012).

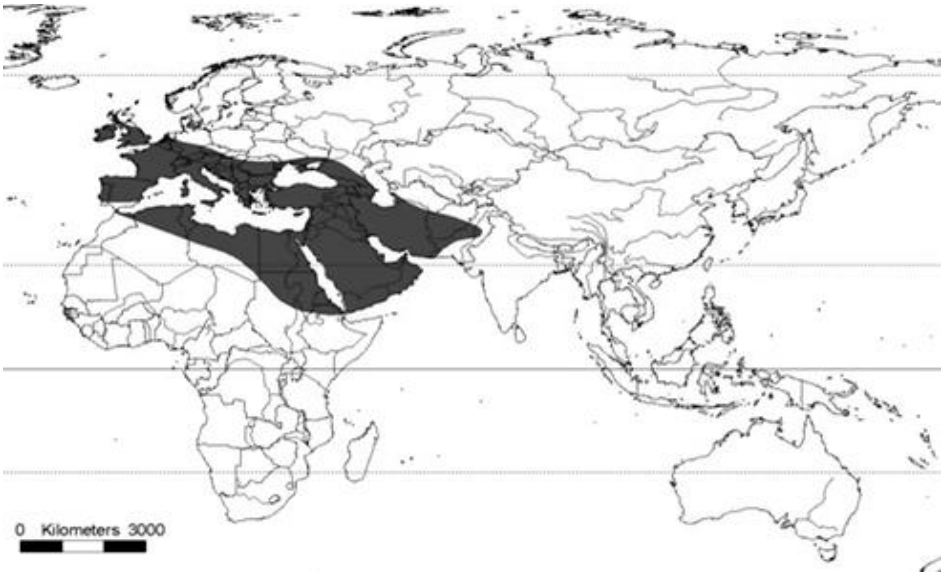


Fig. 1.1 Global distribution of *R. hipposideros* (adapted from GROMS, accessed 8 July 2013).

The populations of *R. hipposideros* have suffered a severe decline in many northern and western European areas (Bontadina et al., 2000). In Belgium, where this species

constituted about 43% of all banded bats during 1939 and 1952, this number has dropped to 3.4% between 1995 and 2008 (Kervyn et al., 2009). In the United Kingdom the species is confined to Western Ireland, Wales and Southwest England (Billington and Rawlinson, 2006) and is considered a rare species (Defra, 2012). In Germany, is presently considered a threatened species and its status is "Critically endangered" (BMU, 2010) because many colonies have become extinct; in fact the total population is estimated between 1800 and 2000 individuals (Seckerdieck et al., 2005). In Switzerland, 39 nursery roosts are known, corresponding to solely 1700 individuals; still, in more recent years there seems to be a positive trend in the number of individuals in the nursery roosts (Bontadina et al., 2000). A similar situation occurs in the Czech Republic, where there seems to be a slight increase in the population trend since 1999, with an actual estimated population size of 3800 individuals (Bontadina et al., 2000). In France, *R. hipposideros* is fairly common and is classified as a "least concern" species (UICN et al., 2009), though there seems to be a decrease in the number of individuals in the northern populations (Bontadina et al., 2000).

In Poland, *R. hipposideros* is considered an endangered species (MoE, 2010b) with the major nursery and winter colonies located in Zbójecka Cave with slightly more than 500 individuals (Sachanowicz et al., 2006).

Little is known about the population trends of *R. hipposideros* in southern European countries. The species seems to be relatively common but, at the same time, there are several reports of threatened colonies. In Spain, *R. hipposideros* has a wide but irregular distribution, and the total size of the Spanish population is unknown (Migens, 2002).

The evaluation of the population trends of *R. hipposideros* in Portugal is undetermined (ICNF, 2012) and the quality of the data on the distributional range of this species is poor (ICNB, 2008). However, it seems to be the species of the genus *Rhinolophus* with the largest population in the country, forming nursery colonies of dozens or, in some cases, of hundreds of individuals, and occurring along the entire Portuguese territory (Palmeirim and Rodrigues, 1992; Palmeirim et al., 1999; Rainho et al., 1998). Nevertheless, it is classified as "Vulnerable" by the Portuguese Red Data Book of Vertebrates (Cabral et al., 2005).

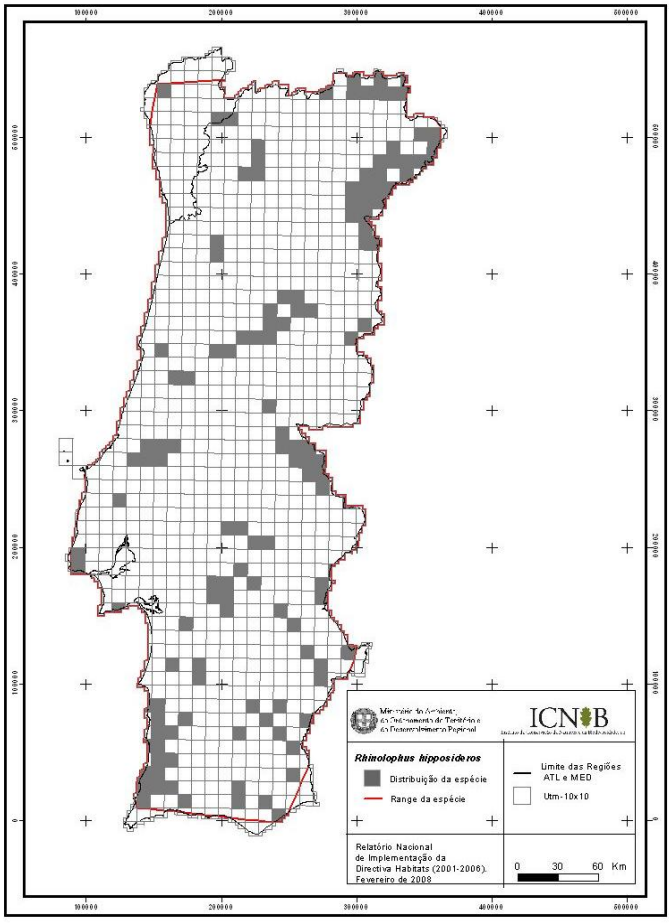


Fig. 1.2 Distribution of *R. hipposideros* in Portugal (ICNB, 2008).

### 1.6. Ecology of *R. hipposideros*

a) Roosting ecology of *R. hipposideros*

*R. hipposideros* seems to be selective in the choice of roosts and the environmental requirements in roost selection seem to be similar throughout the distribution range of this species (Schofield, 1996). This selection is affected by many factors – dimensions of the roost, ease of access, environment around the roost, luminosity, temperature and humidity (Kelleher, 2006).

Hibernation roosts are usually undisturbed sites, with low temperatures and high levels of humidity (MacDonald and Barret, 1993). Webb et al. (1996) showed that *R. hipposideros* hibernates in roosts that range between 2ºC and 13ºC.

Maternity colonies are composed by females, pups of the year and a limited number of males; the size of these colonies varies greatly, from 10 to 500 adults (MacDonald and Barret, 1993). Nursery colonies usually occupy large areas that provide a wide range of microclimates (Freer et al., 1998), because this influences the development of pregnancy and the growth of the juveniles (Lindström, 1999). Typically, pregnant female *R. hipposideros*, and later their young, select areas of the roost with temperatures above 27ºC (Freer et al., 1998). The use of large spaces is also due to the fact that the young bats need to exercise their wings before starting to fly independently, to practice flights inside the roost, and to perform extensive flights out and back into the roost (McAney and Fairley, 1988; Seckerdieck et al., 2005).

*R. hipposideros* also selects higher locations within the roost, usually above 2 m high, probably because these sites are more secure against predators such as stoats, weasels and domestic cats (Freer et al., 1998).

The surrounding landscape also seems to be important to the choice of roosts by *R. hipposideros*. This species rarely crosses open areas and requires high quality habitat around the shelter. In the United Kingdom, *R. hipposideros* prefers roosts surrounded by patches of deciduous and mixed forests and where the different patches are connected by vegetative corridors that protect bats against predators (Schofield, 1996). McAney and Fairley (1988) report that, in Ireland, the habitat around the roost is composed by permanent pastures with fields separated by mixed hedgerows.

*R. hipposideros* forms nurseries in natural underground sites but they also use manmade structures as summer roosts. All across Europe *R. hipposideros* seems to largely use old buildings (Boye and Dietz, 2005; Cabral et al., 2005; Knight and Jones, 2009; Palmeirim et al., 1999; Seckerdieck et al., 2005), often with stonewalls and slate roofs (Schofield, 1996), probably due to the similarity with the natural roost sites.

b) Foraging behaviour and diet of *R. hipposideros*

Many studies have shown that *R. hipposideros* forages on areas of extensive woodland cover or dense scrub, usually located close to suitable roost sites (Bontadina et al., 2002; Reiter, 2004a; Schofield, 1996). In western Ireland, McAney and Fairley (1988) found that *R. hipposideros* forages preferentially on riparian vegetation and over farmyards. In the United Kingdom, this species forages mainly in mixed woodland areas, using also hedgerows and tree lines and, occasionally, in riparian vegetation (Schofield, 1996). Bontadina et al. (2002) confirmed its preference for broadleaf woodlands and, in addition, demonstrated that areas with high habitat diversity are favoured. In their study, they found that some bats also forage in riparian vegetation but no overall selection for this vegetation type was found. In Germany the specie seem select deciduous and mixed woodland to forage (Zahn et al., 2008). In Austria also was found that woodlands are important foraging habitats and any loss of woodland near the roosts can have a negative influence to the colonies (Reiter et al., 2012). In southern Europe, little is known about the foraging habitat preferences of this species.

In central Europe and in the United Kingdom the diet of *R. hipposideros* is composed essentially by small Diptera, Lepidoptera, Trichoptera and Neuroptera (Arlettaz et al., 2000; McAney and Fairley, 1989). To our knowledge, ours is the first study on diet composition and prey selection by *R. hipposideros* in southern Europe.

c) Reproduction of *R. hipposideros*

Reproductive cycles are energetically expensive and the periods in which the different stages occur are dictated by environmental factors such as climate and food availability (Arlettaz et al., 2001; Lučan et al., 2013).

As other rhinolophids of the temperate region, *R. hipposideros* is characterized by seasonal monoestry (Fig. 1.3). This means that this species presents a single breeding season during each year (Rasweiler and Badwaik, 2000). Male and female gamete cycles are typically desynchronized: in males, spermatogenesis begins in the spring/summer and ends in late summer or early fall (Krutzsch, 2000). Females have an extended period of oestrus that

begins in autumn; the mating period occurs in late autumn (Rasweiler and Badwaik, 2000), after which the sperm is stored in the female tract (Hosken, 1997), where spermatozoids are alive but inactive.

To maximize the reproductive success, the period of ovulation is controlled by the female body, and ovulation usually occurs in the spring when the environmental conditions, in terms of temperature and food availability, are best to support the energetically expensive pregnancy (Dietz et al., 2007). Females usually give birth between late June and early July (Reiter, 2004a; Schofield, 1996), when food availability is maximum (Arlettaz et al., 2001; Humphrey, 1975).

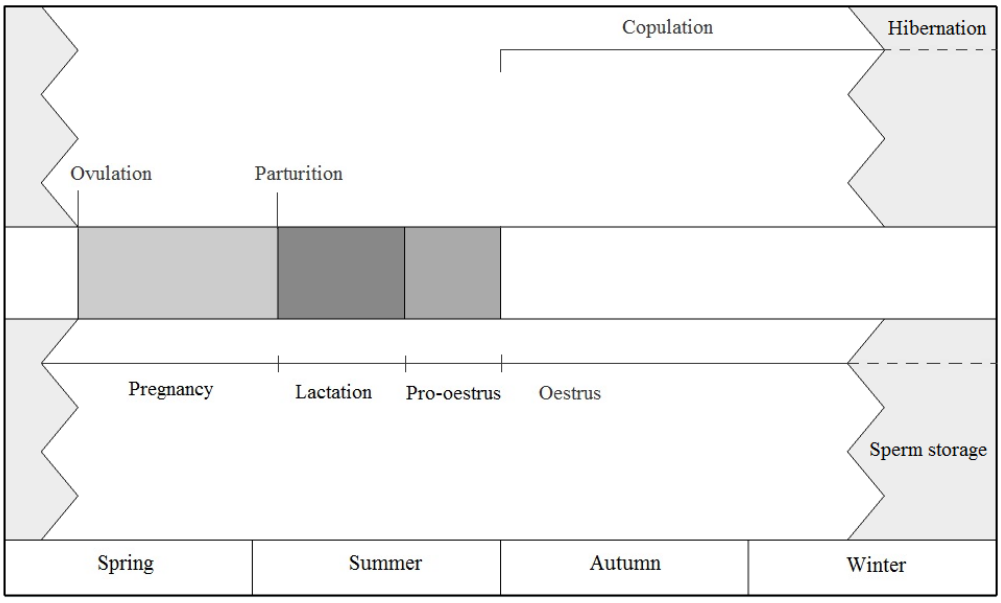


Fig. 1.3 Seasonal reproductive pattern of *R. hipposideros* (adapted from Oxberry (1979)).

At birth pups weight between 1.3 g and 2 g and are completely dependent of their mother, at least until weaning, about four weeks later (MacDonald and Barret, 1993). Besides the provision of milk, young bats need additional maternal care, including sensory stimulation, thermal maintenance and transport (Kunz and Hood, 2000). Just a few days after birth, young bats start to practice flight suspended on their mother and, shortly after, they begin the practice of short flights within the roost. They become completely independent about six to seven weeks after birth (MacDonald and Barret, 1993).

d) Activity patterns

Insectivorous bats of the temperate region normally follow a bimodal pattern of nocturnal activity with a first peak recorded at dusk and a smaller peak before dawn (Erkert, 1982 in Knight and Jones, 2009). These peaks seem to overlap with peaks of abundance of insects at dusk and dawn. Indeed, in Ireland, activity peaks are dictated by ambient light levels (McAney and Fairley, 1988). This seems to be especially relevant to *R. hipposideros* that feeds on crepuscular insects, e.g. many families of Nematocera that present swarming behaviour (Knight and Jones, 2009). This topic is one of the focuses of Chapter 3.

## 1.7. Objectives

As shown above, there is rather good knowledge on the ecology of *R. hipposideros* in north and central Europe. However, generalizations about the roosting and feeding ecology of bats are not straightforward because selective pressures may change between regions (Kunz and Lumsden, 2003). Therefore, specific information about the feeding and roosting ecology of each species in several sites of their distribution range is essential. In this context, the objectives of this study are:

I. To describe the daily and monthly patterns of maternity roost utilization by *R. hipposideros* in a roost of the Mediterranean region and to understand how different abiotic factors affect those patters – Chapter 3.

II. To characterize the diet of *R. hipposideros* of that same roost, to evaluate the abundance and diversity of potential prey in the surrounding area, and to determine the existence of active prey selection – Chapter 4.

# Chapter 2

Study area

# 2. Study area

## 2.1. Location, climate and landscape

The studied colony is located in the Sintra mountain range in the S. Martinho locality, Lisbon district, Portugal (latitude 38°47'45.4452"N and longitude 9°23'51.0972"W; Fig. 2.1).

The region of Sintra has a very specific microclimate resulting from its location and orography (Domingos, 2008): it is a mountainous-forested area bordered on the west side by the Atlantic Ocean. Precipitation is relatively high in the mountains (average: 1006 mm/year), while temperatures are mildly cold (average: 14.8 °C/year; Coutinho et al., 2012; Lousã et al., 2005), originating long winters and relatively short summers.

The flora of the Sintra Mountain is extremely exuberant. In the past the vegetation cover was typically Mediterranean, with *Quercus* spp., *Pinus pinea* and *Ilex aquifolium*. However, currently this vegetation is restricted to some sites because most of the original vegetation was replaced by exotic species of trees (Baltazar and Martins, 2005). Here, the human intervention always had a profound impact on the landscape; the first human activity that contributed to the degradation of the original vegetation were pasture followed by agriculture and tree plantations e.g. *Pinus pinaster* and *Cupressus lusitanica* (Baltazar and Martins, 2005). During the romantic period, in the 19$^{th}$ century, many agricultural fields were transformed into parks, where several exotic and ornamental species were planted, e.g. *Eucalyptus globulus*, *Acacia* spp., *Pittosporum undulatum*, *Hakea salicifolia*, *Hakea sericea* and *Ailanthus altissima*. Some of these have even become invasive.

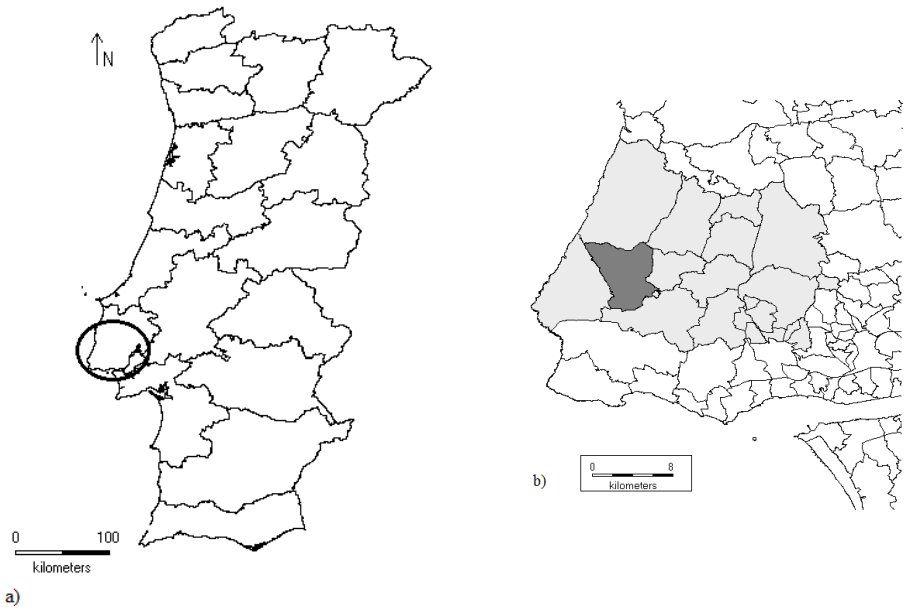


Fig. 2.1 a) Approximate location of the study area in Portugal; b) Sintra Municipality (light grey) and S. Martinho locality (dark grey).

## 2.2. The studied roost

In 2001 a large colony of *R. hipposideros* was discovered in the Monserrate palace, Sintra, harbouring approximately one hundred individuals. In 2008, the palace, which is architectural heritage, suffered restoration works endangering the persistence of the colony. To minimize this potential negative impact, the Cultursintra Fundation, the Sintra Speleological Group and the former Institute of Nature and Biodiversity Conservation, presently, ICNF – Institute for the Conservation of Nature and Forest tried to create similar conditions in an infrastructure of the "Quinta da Regaleira", located at an approximate distance of 2 km from the Monserrate palace (Fig. 2.2). This old infrastructure, called in this work as "ancient roost" (Fig. 2.3a), was built between 1904 and 1910 and was already occupied by a smaller colony of 40 individuals of *R. hipposideros*. The implemented measures began with the installation of one oil heater in the roosting area to try to create temperature conditions similar to those of the Monserrate roost. Then, above the heater,

was placed a shelf close to the ceiling, to further increase the temperature in this area. Remote sensing video cameras were also installed in the roost.

In early summer of 2009 a new space, from now on called "new roost" (Fig. 2.3b) – only 30 meters apart from the "ancient roost" – was handed over by the "Quinta da Regaleira" administration to work solely as a roost for *R. hipposideros* and, eventually, for other species of bats. Here, two oil heaters, one shelf, and four cameras were set in place, and, in 2011 this area was already significantly used by a part of the colony. In 2012 a weather station was set outside the roost. This colony is currently known as one of the largest maternity colonies in Portugal.

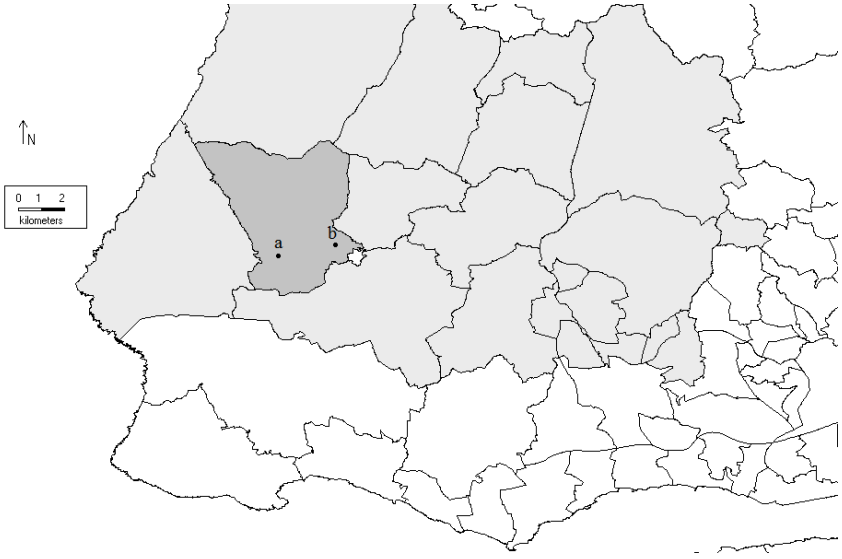


Fig. 2.2 Location of the Monserrate palace (a) and "Quinta da Regaleira" (b) where are located the studied roosts - "ancient roost" and the "new roost".

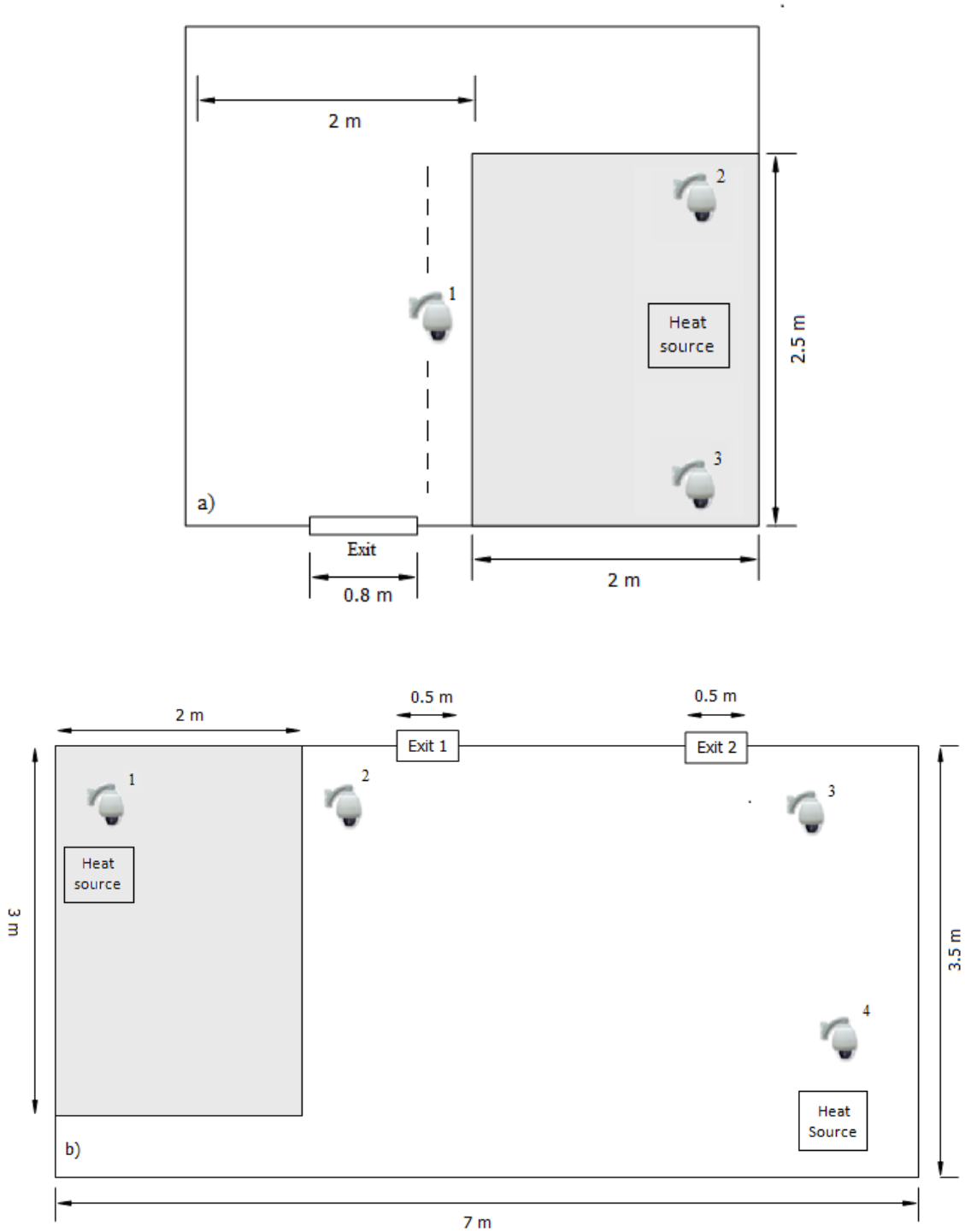


Fig. 2.3 Schematic representation of the studied roosts: a) “ancient roost”; b) “new roost”. The light grey space corresponds to shelves placed in the roost; because oil heaters are set beneath these shelves in both the “ancient roost” and the “new roost”, these are warmer areas. These shelves are placed near the ceiling. The cameras represent the areas that were video recorded. Both roosts are 5 m high.

# Chapter 3

Patterns of use roost by the lesser horseshoe bat (*Rhinolophus hipposideros*) in Sintra, Portugal.

# 3. Patterns of use roost by the lesser horseshoe bat (*Rhinolophus hipposideros*) in Sintra, Portugal.

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## 3.1. Abstract

Despite their capacity to occupy a wide range of habitats and have a large feeding spectrum, almost all microchiropteran bats are exclusively nocturnal. However, the activity patterns throughout the night can differ from species to species and also within the same species from place to place. *Rhinolophus hipposideros* (Bechstein, 1800) has suffered a severe decline in many countries along its distribution area, including in Portugal, where the main cause for decline seems to be the loss of suitable roosts. In this work we aimed to describe the roosting behaviour during spring, summer and autumn of one of the largest maternity colonies of *R. hipposideros* known in Portugal. For this we analysed roost occupancy, activity patterns inside the roost, time of births, and post-natal behaviour. We also tested the influence of ambient factors on the patterns of roost use. All information was obtained through the analyses of videos recorded using cameras placed inside the roost, while climatic data was gathered using a meteorological station. We found that this roost has higher occupation during August and that in October it is only occupied during the night. Females give birth from late May to early June, and in August juveniles seem to become independent and undistinguishable from the adults. This colony presented a

bimodal pattern of activity throughout the study months that seems to be related with sunset and sunrise. In the late summer the time spent outside the roost is longer, suggesting more time spent foraging. Outside ambient temperature was negatively related with roost occupancy whereas pressure, humidity, wind and precipitation were positively related with the number of individuals in the roost, indicating that bats prefer higher temperatures and low humidity/rain, wind speed and pressure to forage.

Key Words: *Rhinolophus hipposideros*; paternal care; roost use.

### 3.2. Introduction

About of 51% of all microchiropteran bat species are listed as either critically endangered, data-deficient, vulnerable, or near threatened (Frick et al., 2010; Hutson, 2001).

Roosts have a fundamental role in the life history of bats and microchiropterans, in particular, show a wide diversity of roost types, such as caves, tree hollows, leaves and manmade structures (Kunz, 1982; Kunz and Lumsden, 2003). So, information on patterns of roost use by bats is fundamental to the knowledge of life history, social systems and roosting and breeding behaviour, helping to develop management plans to secure the survival of bat species (Fenton, 1997).

In Holartic bats hibernation roosts ensure winter survival when thermoregulatory requirements are high and food availability is reduced, also providing protection from predators (Mayle, 1990). On the other hand, summer roosts play an important role in the development of pregnancy and in the subsequent growth of juveniles. Summer roosts are important social units (Boye and Dietz, 2005) and the availability of suitable roosts can promote different degrees of fidelity to these sites (Kunz, 1982). Indeed, in many species, nursery colonies are quite faithful to their location, returning every year to the same site (Lewis, 1995), a tendency called natal philopatry.

Microclimate is one of the most important factors playing a role in roost selection: during pregnancy, female bats need warm roosts to reduce energy costs to maintain

homoeothermy and to accelerate embryonic development (Kerth et al., 2001). Indeed, several studies have demonstrated that temperature defines birth timing, growth rate and the fitness of juveniles and, consequentially, population trends (Dietz et al., 2007; Kerth et al., 2001; Reiter, 2004b).

The lesser horseshoe bat, *Rhinolophus hipposideros*, is the smallest horseshoe bat in Europe (Arlettaz et al., 2000). It weights between 5 and 9 g, head-body length is usually between 37 and 45 mm, and forearm length between 32 and 42.5 mm (MacDonald and Barret, 1993). The mating of this species usually occurs during the autumn, between September and November but can continue during hibernation, even with torpid females (Krutzsch, 2000; MacDonald and Barret, 1993). Both males and females are usually sexually mature when they reach one year of age. As many other Holartic bats, e.g. *Rhinolophus ferrumequinum* (MacDonald and Barret, 1993) and *Myotis lucifugus* (Fenton and Barclay, 1980), each female gives birth to a single young per year and juveniles become completely independent at six or seven weeks of age (MacDonald and Barret, 1993).

One of the largest known breeding colony of *R. hipposideros* in Portugal occupies an ancient manmade structure within the "Quinta da Regaleira", in Sintra (Fig. 3.1). To guarantee the continuous use of this breeding roost by the colony, where it is free from human disturbance and even welcomed by the owners, since 2002 the Sintra Speleological Group (AES) have been monitoring the roost and in 2008 was started monitoring through a remote video recording system. Still, regular and continuous recordings only started in 2011.

This species seems to need large roosts with different microclimates during the breeding season though warmer roosts are clearly preferred (Schofield, 1996). Across the years, pregnant females and juveniles seem to select different areas within the roost and this choice seems to be correlated with external temperatures once the roost is artificially heated using an oil heater and so the temperatures never went below 16ºC.

However, patterns of roost use also depend on external environmental conditions. Many studies demonstrated that low temperatures may delay, reduce or avoid the foraging periods of many insectivorous bat species (Anthony et al., 1981; Catto et al., 1995; Maier, 1992). High levels of humidity have been reported to provide good conditions for bats to forage because high levels of humidity provide higher abundance of insects (Roche, 1997).

Precipitation and wind are also considered important factors affecting the foraging period of bats, though literature is not consistent on the direction of the trends. For instance, the activity of long-tailed bats, *Chalinolobus tuberculatus* in New Zealand, does not seem to be seriously altered by moderate rain or strong winds (Griffiths, 2007). However, strong wind seems to inhibit foraging activity both during the summer and winter in some species (Avery, 1985; O'Farrell et al., 1967; Russo and Jones, 2003). In a study done in Ireland, the activity of *R. hipposideros* seems to be negatively affected by heavy rain (McAney and Fairley, 1988), but information on how other climatic variables influence the activity patterns of this species is still lacking.

The main goals of our study were:

i. to define monthly patterns of occupancy and activity of adult and juvenile *R. hipposideros* in the Sintra roost using video recordings;
ii. to determine the timing of foraging periods and the patterns of nocturnal occupation of the roost;
iii. to define the timing of births and to describe pup behavioural patterns along the first weeks of life; and
iv. to examine how external environmental factors influence the patterns of roost occupancy, and, simultaneously, the foraging activity by *R. hipposideros*.

## 3.3. Materials and Methods

### 3.3.1. Study Area

This study was conducted in Sintra, Portugal (Fig. 3.1) in a maternity colony roosting in an ancient infrastructure of "Quinta da Regaleira". This colony has been consistently monitored since 2002 and initially was composed by approximately 40 individuals. One other colony located in the Monserrate palace at about 2 km of the "Quinta da Regaleira"

was in risk due to restoration works of the palace. So, in 2008 an effort was done at "Quinta da Regaleira" to attract this second colony – the roost was safeguarded from human disturbance and was artificially heated. Because no bats were marked, there is no way of knowing if the increase in the number of bats in "Quinta da Regaleira" was due to the migration of bats from the Monserrate colony. Nonetheless, "Quinta da Regaleira" currently represents one of the major known breeding colonies of *R. hipposideros* in Portugal.

The area surrounding the "Quinta da Regaleira" roost is characterized by typical Mediterranean woodland vegetation such as, *Quercus* spp., *Pinus pinea* and *Ilex aquifolium*, though some plantations of *Pinus pinaster* and *Cupressus lusitanica*, as well as many exotics species as *Eucalyptus globulus*, *Acacia* spp., *Pittosporum undullatum*, *Hakea salicifolia*, *Hakea sericea* and *Aillanthus altissima* are also present. This dense vegetative cover is probably a reflex of the specific microclimate of the Sintra mountain range. Here, high relative humidity levels, mildly cold temperatures (average: 14.8$^{o}$C/year) and significant levels of rainfall for a Mediterranean region (average: 1006 mm/year(Coutinho et al., 2012; Lousã et al., 2005) are common all year round, though rainfall is more frequent during the winter.

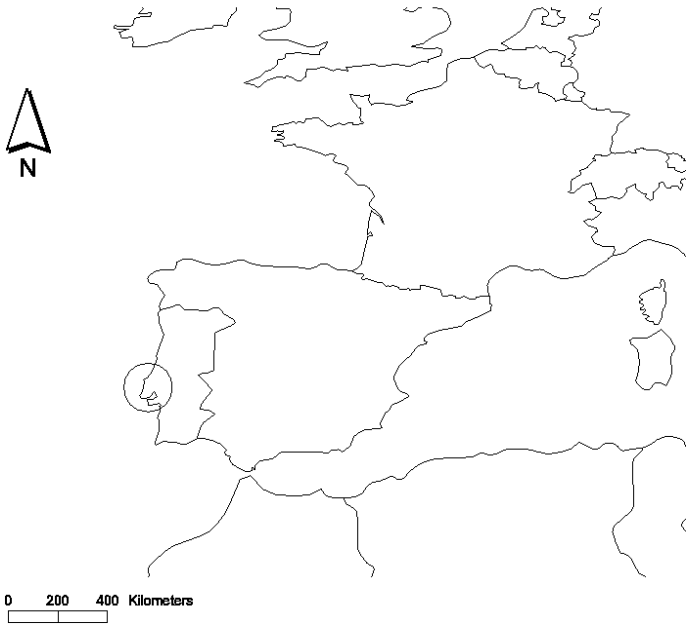


Fig. 3.1 Approximate location of the study area (Sintra, Portugal).

### 3.3.2. Analysis of the video recordings

We analysed video recordings of the roost from March to October of 2010 and 2012. At the beginning of March, females start to settle in the roost and gradually leave from September to October, when the roost is mostly occupied during the night period. The areas captured by the cameras not cover all parts of the roost but captures the most part of the colony. Because some of the equipment experienced technical problems and image capture was not possible for some days, we chose to analyse only days with records of the whole 24 hours in all the main cameras of the roost. From March to September 2010 we gathered recordings from only two cameras, and also from a third camera in October (Fig. 3.2a); in 2012 we gathered images from three cameras in the main division of the roost and from another one in a contiguous room of the roost which had very little occupation (Fig. 3.2b). We analysed images between September and October of 2010 and 2012 but we use only 29 days from 2010 and 69 days from 2012. The table 3.1 represent the number of days that was possible use to describe the occupancy and activity of the studied roost.

Table 3.1 Number of days used to characterize the occupancy and activity in the studied roost.

| **Year** | **Month** | **Number of days** |
|---|---|---|
| **2010** | July | 7 |
| | August | 11 |
| | October | 11 |
| | **Total** | 29 |
| **2012** | March | 4 |
| | April | 29 |
| | May | 21 |
| | June | 1 |
| | July | 3 |
| | August | 6 |
| | October | 5 |
| | **Total** | 69 |

In these two years the colony did not occupy the exact same place. During 2010 it occupied mostly one building, from now on designated as “ancient roost” and in 2012 it started to move to another infrastructure about 30 meters away of the previous one designated as “new roost”. Both roosts were artificially heated, as already mentioned above.

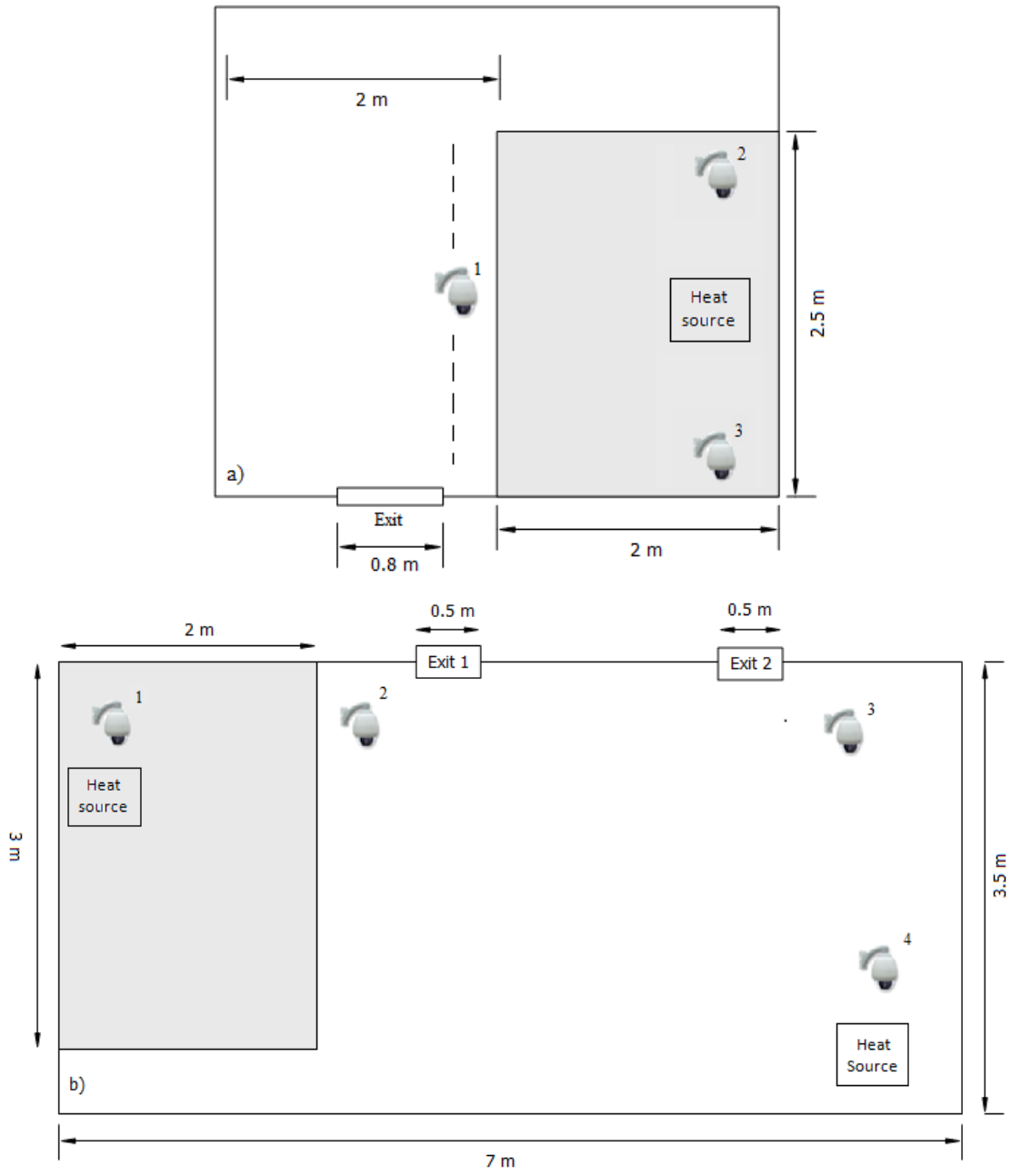


Fig. 3.2 Representation of the studied roosts. a) “ancient roost”; b) “new roost”. The light grey space corresponds to shelves placed in the roost; because oil heater are set beneath these shelves in both the

"ancient roost" and the "new roost", these are warmer areas. These shelves are placed near the ceiling. The cameras represent the areas that were video recorded. Both roosts are 5 m high.

To describe the occupancy of the roost we counted the number of adults and juveniles in the roost, and to describe roost activity we counted the number of flights inside the roost along the day using an approach identical to that used by Weinbeer et al. (2006).

### 3.3.3. Climatic variables

Data regarding temperature, pressure, wind velocity, humidity and rainfall outside the roost was obtained from the meteorological station of Mira-Sintra (http://www.wunderground.com/).

### 3.3.4. Data analyses

To try to understand how the climatic variables affected the use of the roost by *R. hipposideros* we used Generalized Linear Models (GLM), using the number of individuals in the roost as the response variable. Temperature, precipitation, humidity, pressure and wind speed were defined as independent predictor variables. These models were developed using data from the months with higher occupancy levels, in particular July and August 2010 and June, July and August 2012. Analyses were done in R version 3.0.1 (R Core Team 2013).

## 3.4. Results

### 3.4.1. Structure of colony

The colony is almost exclusively composed by *R. hipposideros*, but there were some sporadic visits of *Plecotus* sp. in October 2010. When this happened, it was possible observe some distress of the individuals of *R. hipposideros* that increased the number of flight activity inside the roost and often avoided the proximity with *Plecotus* sp.

### 3.4.2. Monthly patterns of roost occupancy

The number of individuals that occupied the roost changed among the years. Figure 3.3 shows the variation in the number of individuals per month during the year 2010 in the "ancient roost" and also in the "new roost" in 2012.

During daytime, the number of individuals in the roost was more stable when compared with the night.

During the spring of 2012 the number of individuals that occupied the roost was very low, with a maximum monthly mean of 30 individuals (st.dev. = 19). During the summer months, it was possible to distinguish more individuals inside the roost during 2012 than for the same period of 2010. So, in June, July and August 2012, 62 (st.dev. = 0), 106 (st.dev. = 15) and 117 (st.dev. = 29) bats were registered in the roost, respectively (see tables 7.1, 7.2 and 7.3 in the appendix).

In October, the only truly representative month of the autumn season, more individuals were recorded in 2010 than in 2012. During this period the roost was only occupied during the night and the monthly mean number of individuals inside the roost reached 118 individuals (st.dev. = 21).

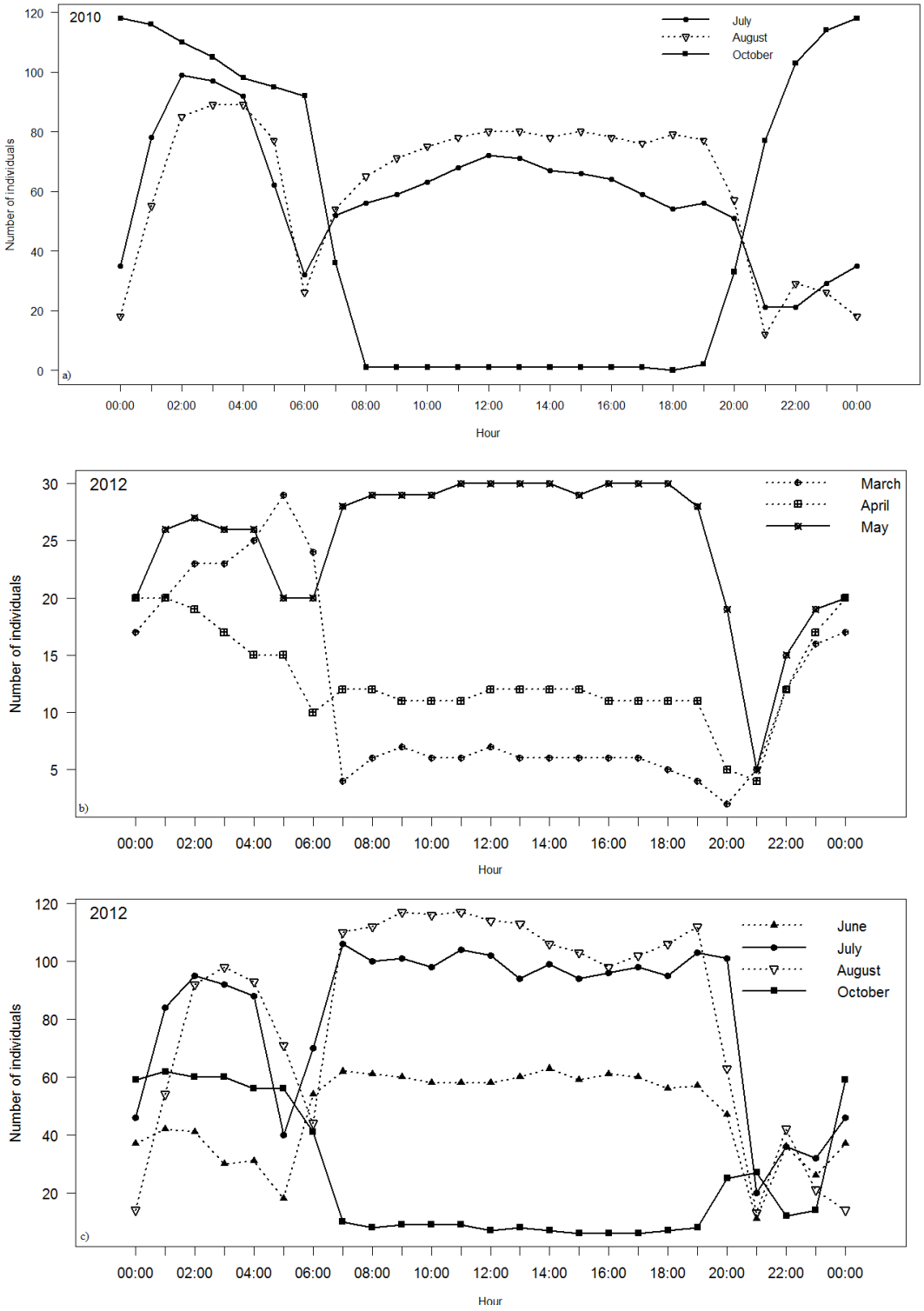


Fig. 3.3 Average number of individuals that occupied the roost in a 24h-cycle during the 2 years: a) year of 2010 in the "ancient roost"; b) and c) year 2012 in the "new roost". Due to technical problems, image capture was not possible for some days, so we decided to analyse only those days with video records for the whole 24 hours in all the main cameras of the roost. The graphs match to the analysis of 21 days from 2010 corresponding to days during the months of July, August and October and 65 days from 2012 corresponding to days during to the months of March, April, May, June, July, August and October.

During July and early August it was possible to recognize juvenile bats (Fig. 3.4). After the second week of August it became very difficult to distinguish juveniles from adults using video recordings.

In July 2010 was possible to count more individuals in the roost than in August 2010. However, in 2012 the number of identifiable juveniles was higher in August than in July. The maximum monthly mean of identifiable juveniles occurred in 2010 (average: 25; st.dev = 12; see table 7.4 in the appendix).

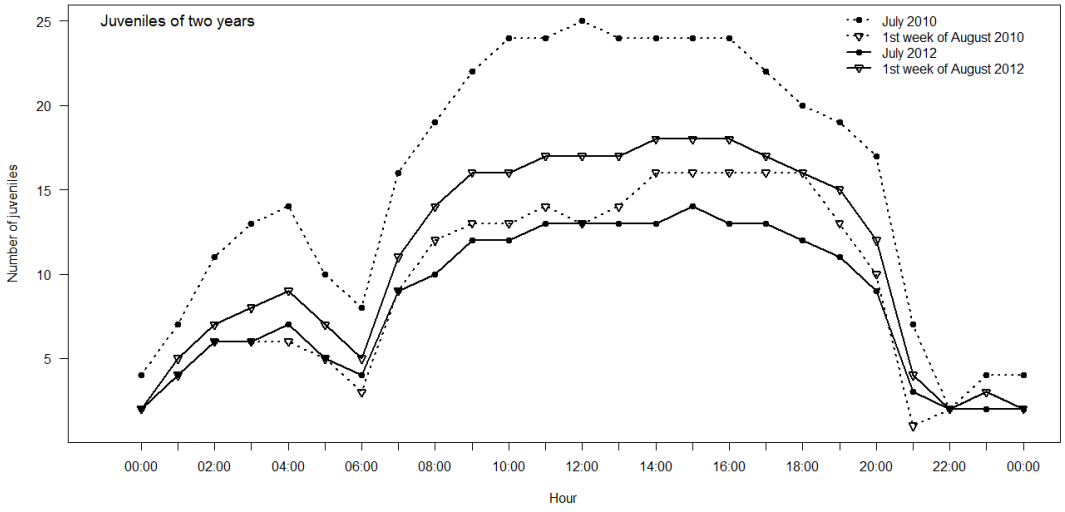


Fig. 3.4 Average number of juveniles that occupy the roost during 2010 and 2012.

### 3.4.3. Foraging periods

#### a) Time of foraging periods

*R. hipposideros* seems to have two main foraging periods during the night (Fig. 3.3) even though the timing of these periods has changed between months and years.

In the summer months of 2010, the first foraging period occurred around 22h00 in July and 21h00 in August and the second foraging period occurred around 06h00.

During March 2012 the foraging periods occurred at 20h00 and 07h00. Between April and August the first foraging period usually occurred at 21h00 and the second period changed between 05h00 and 06h00.

In October 2010 and October 2012, few individuals stay in the roost during daytime and the roost was used mainly during the night. The first foraging period occurred before 19h00, while the second occurred slightly after 08h00 in 2010 and 07h00 in 2012.

b) Relation with sunset and sunrise

Foraging periods seem to be closely related with light intensity (Fig. 3.5). In general they occurred slightly after sunset or before sunrise. In August 2012 there was a higher discrepancy between sunset and the first hunting period.

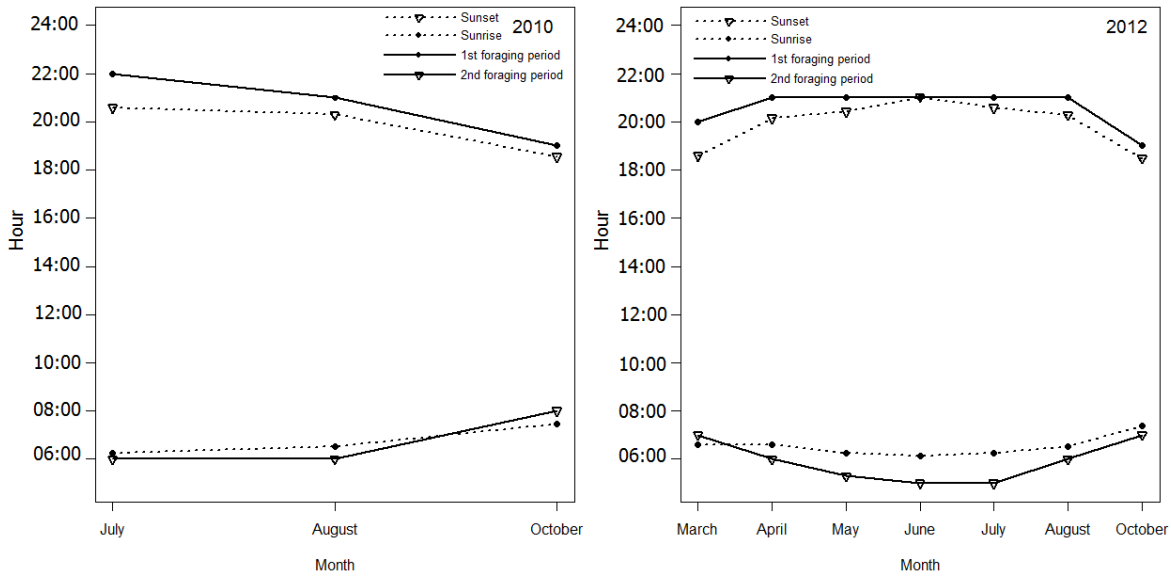


Fig. 3.5 Foraging periods and time of sunrise and sunset during the studied months of 2010 and 2012.

### 3.4.4. Nocturnal occupancy of the roost

When we focus on the time between the first and the second foraging periods (Fig. 3.6), it is possible to see that, over the course of the months, bats spent more time outside the roost; a third peak, occurring, after the first foraging period started to become more obvious in July and August.

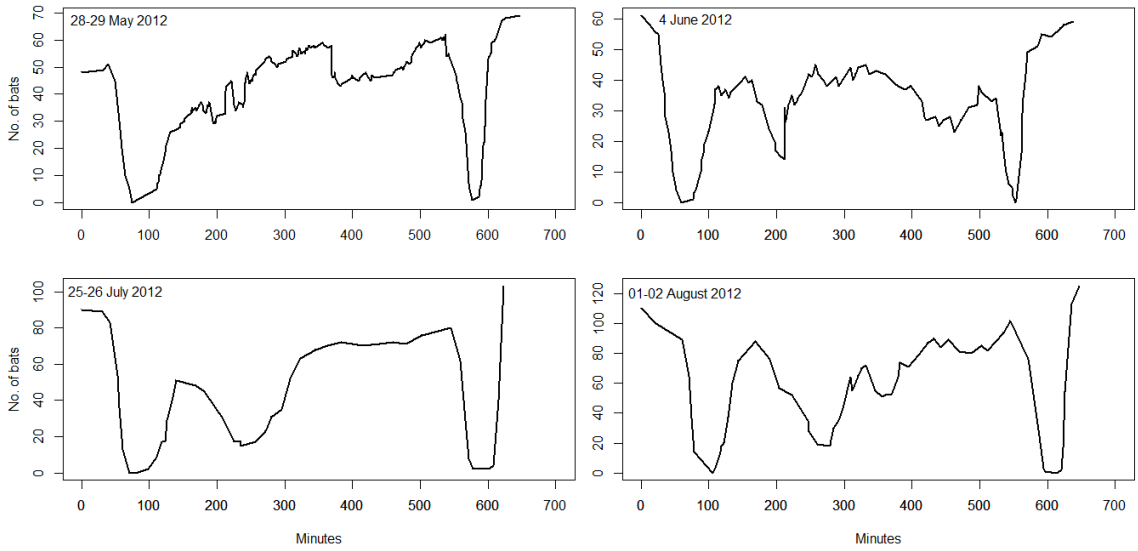


Fig. 3.6 Changes on the activity pattern of roost use throughout the months, between the first and the second hunting period.

### 3.4.5. Monthly activity within the roost

#### a) Flight activity

Flights within the roost seem to be correlated not only with the season but also with the hour of the day. In fact, bats were much more active inside the roost during the night and this was especially marked in July and August (Fig. 3.7). There seems to be two main activity peaks along the night correlated with the foraging periods (Fig. 3.3).

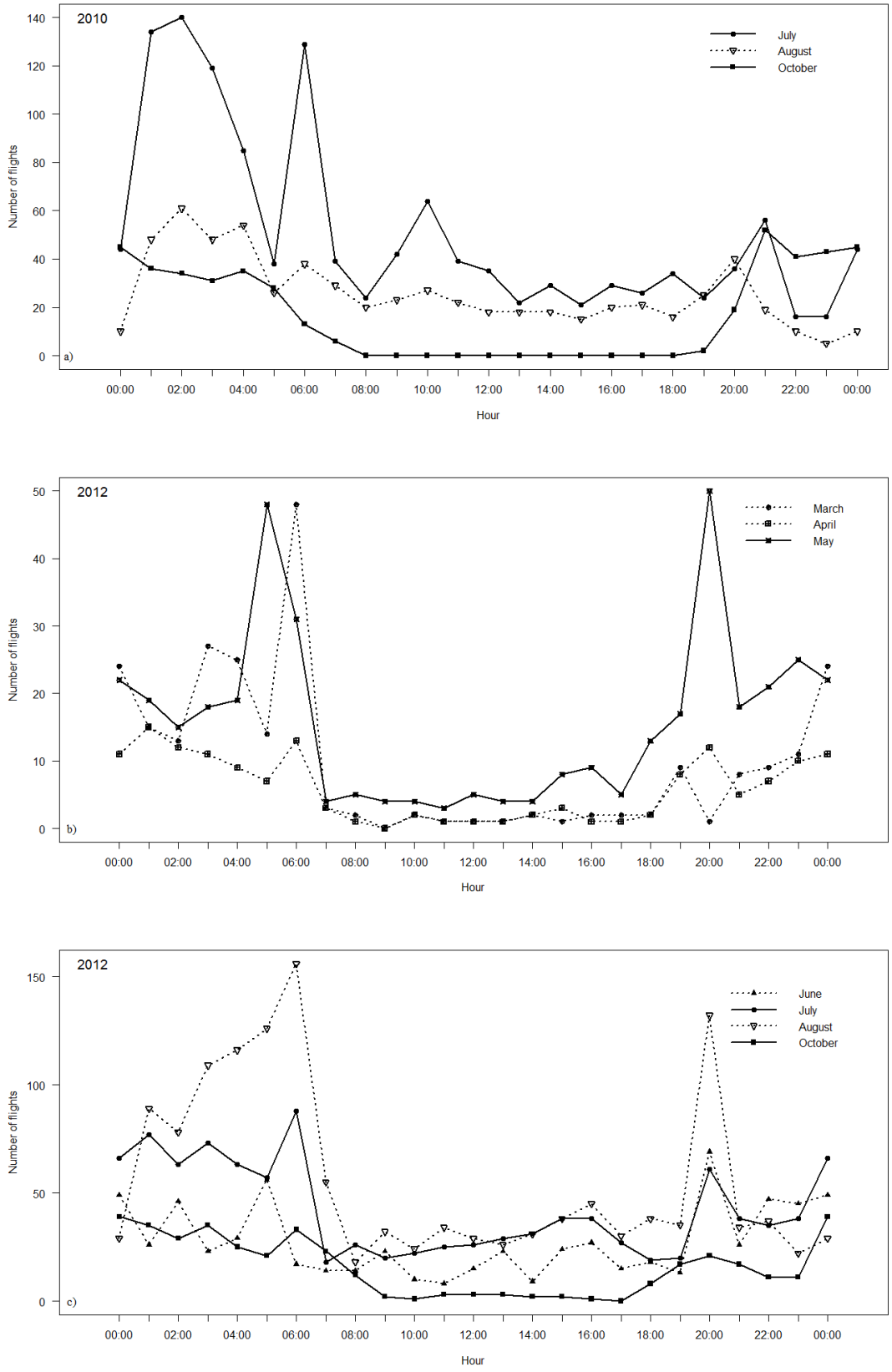


Fig. 3.7 Number of flights in the roost during the 2 years: a) 2010, ‘ancient roost’; b) 2012, ‘new roost’ and c) 2012, ‘new roost’.

b) Juvenile training flights

In July the juveniles started to practice short flights inside the roost and, a few days later, they began to leave the roost by themselves. In July 2010 and 2012 the number of training flights in the roost was very uneven along the day, with some evident activity peaks (Fig. 3.8). In August the number of training flights decreased markedly and it stopped to be possible to distinguish peaks of juvenile activity, mainly because many juveniles became undistinguishable from adults.

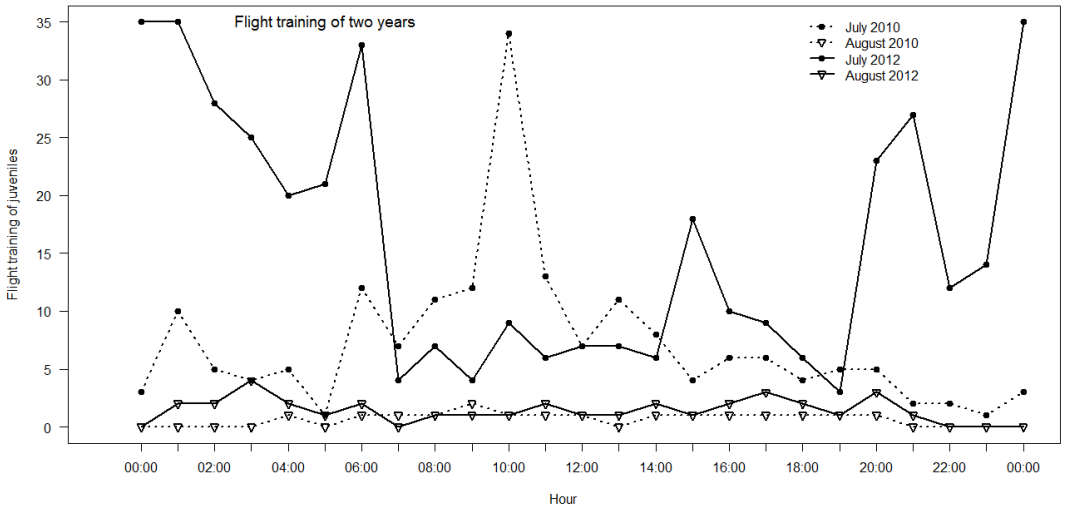


Fig. 3.8 Number of training flights done by juveniles inside the roost in 2010 and 2012.

3.4.6. Births and post-natal behaviour

a) Time of births

Though we have never registered a birth with the cameras, we considered the timing of the first birth when the first pup was recorded in the roost. Between 2010 and 2012 the first pup recorded was only one week apart, in May 27$^{th}$ in 2010 and June 5$^{th}$ in 2012.

b) Post-natal behaviour

Only one day after the first registered pup of 2010, it was possible to observe a pup suspended alone in the ceiling of roost; in the second day a pup was detected suspended in its mother. The first flight attempts were registered four days after the first recorded birth, done by pups suspended in their mothers.

When female bats left the roost to forage, they either carried their young or left them somewhere outside the monitored zone. There does not seem to be any specific location to leave young bats, such as nurseries, as occurs with other bat species, because young *R. hipposideros* usually roosted alone.

Adult females made sporadic visits to the pups left in the roost during the foraging periods.

### 3.4.7. Climatic variables affecting roost occupancy during the night

The best model to explain roost occupancy overnight during summer months, July and August of 2010 and June, July and August 2012, included all variables: temperature, pressure, humidity, wind, and precipitation (Table 3.2). Temperature was negatively correlated with the occupancy of the roost during the night while pressure, humidity, wind and precipitation were positively correlated with the occupancy of the roost during the night (Table 3.3).

Table 3.2 Summary of the best GLM explaining roost occupancy overnight during the months showing the highest occupancy rates. The best model is highlighted in grey.

| | **Deviance** | | | |
|---|---|---|---|---|
| **Model** | **Null** | **Residual** | **AIC** | **wAIC** |
| Null | 83684 | 83684 | 85069 | 0.00E+00 |
| Temperature+pressure+humidity+wind+ precipitation | 9698.9 | 8099.7 | 9496.9 | 1.00E+00 |
| Temperature+pressure+wind+ precipitation | 9698.9 | 8134.5 | 9529.7 | 7.54E-08 |

Table 3.3 Parameters for the variables included in the best GLM explaining roost occupancy overnight during the months showing the highest occupancy rates.

| Models/Variables | Estimate | Std.Error | z value | Pr(>\|z\|) |
|---|---|---|---|---|
| Intercept | -27.293106 | 3.353643 | -8.138 | 4.01E-16 |
| Temperature | -0.051682 | 0.006355 | -8.132 | 4.21E-16 |
| Pressure | 0.030638 | 0.003246 | 9.440 | <2.00E-16 |
| Humidity | 0.007945 | 0.001351 | 5.882 | 4.05E-09 |
| Wind | 0.022881 | 0.001149 | 19.908 | <2.00E-16 |
| Precipitation | 0.456549 | 0.052722 | 8.660 | <2.00E-16 |

## 3.5. Discussion

Despite some technical problems with the cameras, which reduced the number of available recordings, our data allowed a reasonable idea on the factors influencing roost occupancy and on the patterns of activity of *R. hipposideros* in the studied nursery roost. In addition, using video recordings, no disturbance was made during this very sensitive time of the life-cycle of bats, guaranteeing that the most natural possible behaviours were registered.

### 3.5.1. Foraging periods and nocturnal occupancy of the roost

*R. hipposideros* seems to present a bimodal pattern of activity that varies seasonally. In fact, the availability of aerial insects, daylight-length (Catto et al., 1995; McAney and Fairley, 1988), temperature (Lacki, 1984; O'Donnell, 2000; Russ et al., 2003; Russo and Jones, 2003), and other climatic factors as rainfall (Weinbeer et al., 2006), relative humidity (Lacki, 1984) and wind speed (Russ et al., 2003; Russo and Jones, 2003) seem to have an important role in the activity patterns of insectivorous bats.

Some studies show that insectivorous bats present this bimodal pattern due to the exploitation of peaks of activity of aerial insects: a first peak in the number of insects usually occurs after dusk and a second just before dawn (Jones and Rydell, 1994; Rautenbach et al., 1988; Swift, 1980). As insect activity seems to decline in the middle of

night, apparently caused by a drop in ambient temperature (Rydell, 1992), insectivorous bats do not have any advantage in a continued foraging activity all night long (Jones and Rydell, 1994; Rautenbach et al., 1988), because this would mean a waste of energy (Jong and Ahlen, 1991; Kunz, 1973b; Swift, 1980). According to Schoener (1971), under the light of the optimal foraging theory, the optimal diet is one which provides one way to obtain the greatest net energy per unit feeding. Because the availability of insects after dusk and before dawn is high, during this period bats are not required to spend too much energy to search and capture prey. Therefore they take advantage of this abundance of prey to yield maximal net energy gains and to minimize the energetic expenditures when searching for food.

From June to August a third activity peak is also noticeable indicating that bats spend more time foraging during the night in later summer. This behaviour is probably due to increased energy demands, resulting from milk production during the lactation period (Catto et al., 1995) followed by the need to restore fat reserves for winter. Complementarily, it may be due to the fact that summer nights are warmer, promoting increased prey activity for longer periods, which bats tend to explore.

### 3.5.2. Monthly activity in the roost

Flight activity within the roost varies along the day and seems to have a strict relation with the foraging periods. Increased activity before the first foraging period is probably related to the fact that adult bats need to check levels of light intensity before leaving the roost (Griffiths, 2007; McAney and Fairley, 1988; Seckerdieck et al., 2005), and thus perform several flights exiting and entering the roost before they definitely departure to forage (McAney and Fairley, 1988). Light sampling plays an important role in the regulation of the nocturnal activity of several bat species and it is also one way of bats avoiding predators that need of at least some light to capture their prey (Griffiths, 2007; Isaac and Marimuthu, 1993).

Daytime flights inside the roost seem to happen as a way to change roosting location and, in juveniles, for flight practice. It may also happen as a response to intruders as one way to

protect the colony; in fact, though happening a very few times, we registered avoidance behaviour in *R. hipposideros* when individuals of *Plecotus* sp. entered the roost.

Flight is an energetically expensive activity (Maier, 1992); the energy expended in flight by bats seems to be positively related with body mass (Winter and Von Helversen, 1998), so it is expected that bats require more energy when females are pregnant or have to carry their pups. During the lactation period, few pups were registered in the roost during the foraging periods of the adult females, suggesting that they were carried by them. A small number of females left their pups in the roost during a few nights, but they do not seem to show any preference by the place where to leave the young bats, a behaviour already described by Schofield (1996) in this species and by Sano (2000) in *Rhinolophus ferrumequinum* in Japan.

### 3.5.3. Time of birth and post-natal behaviour

During the two years of the study, 2010 and 2012, births happened relatively early, when compared with the patterns described in other locations (Ifrim, 2007; Reiter, 2004b; Schofield, 1996). In our study, births started in 27$^{th}$ May in 2010 while in Wales and in Romania births only started in the end of June and in the second half of July, respectively (Ifrim, 2007; Schofield, 1996).

This is surely due to the fact that timing of parturition is strongly influenced by environmental factors, including climate and food availability. In lower latitudes, ambient temperature, and consequently prey availability, start to increase sooner in the year that in northern areas, explaining this asynchrony in birth timings between our study and those of Ifrim (2007) and Schofield (1996). Food availability is indeed a chief factor regulating the timing of parturition (Arlettaz et al., 2001) and late parturition may negatively affect growth and survival of young bats (Ransome and McOwat (1994). *R. hipposideros* in Sintra have thus more time to store fat reserves for hibernation, which may increase the probability of survival and breeding during their first year when compared with pups that are born later in the summer or from higher latitudes.

### 3.5.4. Environmental factors affecting roost occupancy overnight

Manmade structures usually exhibit a marked variation in climatic parameters, especially temperature and relative humidity, in contrast to the constancy presented by underground roosts (Kunz, 1973a).

However, though this roost is located in a manmade infrastructure, the internal temperature is somewhat controlled by the presence of heaters that provided different microclimates but not present significant variations inside the roost. So, the variation of the temperature is probably not a factor strongly influencing bat activity in the studied colony.

Life strategies of bats are regulated by many factors e.g. prey availability, and moonlight but climate is surely among the most important as it can affect bats directly, inhibiting some activities such as flight, foraging and mating. Indeed, it is know that bats have minimum ambient temperatures to forage, and during cold nights it has been shown that the length of the foraging periods decreases, so bats spend more time inside their roosts (Roche, 1997). Even in higher latitudes, where bats are more adapted to cold nights, there seems to be a correlation between bat activity and ambient temperature; for example, in southern Sweden between May, June and July, which corresponds to the breeding season of *Eptesicus nilssoni* (Rydell, 1989, 1993), it has been shown that it only forages with temperatures above 10ºC (Rydell, 1989).

However, ambient temperature also influences bat activity indirectly by affecting the abundance and the activity of arthropods (Anthony et al., 1981; Rydell, 1989; Taylor, 1963), which are the main components of the diet of insectivorous bats.

We found that lower humidity levels lead to an increase of foraging activity, or at least to more individuals outside the roost. These results are not consistent with other studies, such as that of Lacki (1984) with *M. lucifugus* in southeast Ohio. Males of *M. lucifugus* showed higher activity levels, by being captured more than expected, during nights with higher ambient humidity. Indeed, flying with higher levels of humidity seems to be a behavioral strategy to reduce excess water loss and, as insects have higher level of activity under these conditions, bats need to spend less time to search and feed on them (Roche, 1997). A possible explanation for a decreasing activity with moister nights is the fact that, in our study area, nights are frequently very humid and the relation between bat activity and this

variable may be unimodal rather than linear, i.e., activity is positively related to humidity only up to some point, after which it becomes too humid and bat activity decreases. However, our data did not allow us to test this hypothesis.

*R. hipposideros* clearly decreased the foraging activity with increasing rain and wind speed. In this case our results seem to be consistent with other studies that argue that strong winds tend to challenge bat flight. In Italy, Russo and Jones (2003) showed that the activity of *Myotis daubentonii* and *Myotis capaccinii* were clearly reduced by strong winds. Other studies, however, argue that this negative correlation is caused by the effect of wind on insect availability, because strong winds preclude the flight of insects (Peng et al., 1992) making them unavailable to bats that forage on flying arthropods.

The influence of rain in bat activity is not straightforward. In southern England and in New Zealand no relationship was found between moderate rain and activity in *Eptesicus serotinus* and *C. tuberculatus*, respectively (Catto et al., 1995; Griffiths, 2007). In other studies rain seems to promote a reduction of bat activity or it at least delays roost departure (Erickson and West, 2002; Weinbeer et al., 2006) as seems to occur with *R. hipposideros* in Ireland (McAney and Fairley, 1988). In Panama, flight time per night in *Macrophyllum macrophyllum* was clearly affected by rain when compared with nights without rain, as bats spent less time hunting during periods of heavy rain (Weinbeer et al., 2006). In Poland, a negative effect between flight activity and rainfall was also found in *E. serotinus* and *Nyctalus noctula* (Ciechanowski et al., 2007) .

### 3.6 Conclusions

The peak of occupation of the studied roost occurred during the July and August. During the two years of study, parturition time was between late May and early June. The foraging activity of *R. hipposideros* seems to be closely related to light intensity, and is maximum near sunset and sunrise. However, foraging activity of *R. hipposideros* seems to be also determined by other factors such as temperature, wind speed and precipitation.

# Chapter 4

Prey selection by *Rhinolophus hipposideros* (Chiroptera, Rhinolophidae) in a modified forest in Southwest Europe.

# 4. Prey selection by *Rhinolophus hipposideros* (Chiroptera, Rhinolophidae) in a modified forest in Southwest Europe.

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## 4.1. Abstract

The knowledge of the diet of endangered species provides information on ecology and behaviour that is essential for the adequate management of their populations. Population decline may be indeed associated to diet specialization and unavailability of feeding resources, especially in modified environments. We studied the diet and prey selection of *Rhinolophus hipposideros* (Bechstein, 1800) in a modified Mediterranean landscape in Portugal, where this species is classified as vulnerable. Diet composition was evaluated by analyzing faecal pellets collected in a breeding colony of *R. hipposideros*, and the available arthropods were sampled using a light trap set outside the roost. The most consumed prey were Lepidoptera, Tipulidae (Diptera), Hymenoptera and Neuroptera. Arthropod diversity remained fairly constant in the diet, but not in terms of prey availability. Our results show that *R. hipposideros* is a generalist species that feeds on a great number of preys. However, our data suggest that, within the broad dietary niche, *R. hipposideros* is not opportunistic, actively selecting Diptera as a preferred food item.

Key Words: diet, Mediterranean forest, prey selection, *Rhinolophus hipposideros*.

## 4.2. Introduction

The lesser horseshoe bat, *Rhinolophus hipposideros*, is the smallest European horseshoe bat (MacDonald and Barret, 1993). Globally, it is considered a "Least Concern" species according to the IUCN Red List, though it is recognized that many populations are decreasing in several regions throughout its distribution. Since the 1950s, this species has suffered a severe decline in most of Western and Central Europe (Bontadina et al., 2001). Presently, at the European level, it is considered an endangered species that is threatened by the degradation of autochthonous woodlands, which are key foraging habitats for *R. hipposideros* (Bontadina et al., 2002; Motte and Libois, 2002; Reiter, 2004a; Zahn et al., 2008). Few studies have investigated the habitat preferences of *R. hipposideros* in the Mediterranean region, where it seems to prefer Mediterranean and sub-Mediterranean woodlands and Mediterranean macchia for hunting (Russo and Jones, 2003).

Though not an exclusively cave-dwelling species, in Portugal, where it uses caves and mines as well as abandoned buildings, it is threatened not only by the destruction of roosts and the blocking of roost entrances in the case of underground sites, but also by reckless restoration of abandoned houses (Cabral et al., 2005; Palmeirim and Rodrigues, 1992; Rainho et al., 1998).

As in the rest of Europe, serious degradation of native forests and scrubland, where *R. hipposideros* prefers to forage (Rainho, 2007), has also contributed to its decline in Portugal (Cabral et al., 2005). Due to these threats and because the Portuguese population is small and has shown a continuous decline in the number of mature animals, it is classified as "Vulnerable" by the Portuguese Red Data Book of Vertebrates (Cabral et al., 2005).

According to Bontadina et al. (2002), the average home-range of *R. hipposideros* is 600 m and the maximum distance recorded to the roost is 4.2 km; therefore, it becomes clear that the conservation of this small bat depends on a fragile balance between the availability of adequate roosts and the availability of foraging areas and food items in the vicinity of those roosts.

One of the largest nursing colony of *R. hipposideros* in Portugal is located in the Sintra mountain range, which originally harboured typical Mediterranean *Quercus* forests, but has now been severely modified by the introduction of exotic species such as *Eucalyptus*

*globulus*, *Acacia* spp., *Pittosporum undullatum*, *Hakea salicifolia*, *Hakea sericea* and *Aillanthus altissima*. So, the conservation of this important nursing colony is related not only to the preservation of its roost but also on the foraging resources it depends on. Indeed, the persistence of *R. hipposideros* in highly fragmented Mediterranean landscapes probably depends on an ability to adapt its diet to new and modified habitats. In this context, information on the dietary patterns of *R. hipposideros* in human-modified habitats can help to determine how specific land-uses outside well-preserved areas may be adjusted to enhance their value as foraging habitat.

It is known that habitat and diet specialization are two of the main factors influencing extinction risk (Owens and Bennett, 2000; Purvis et al., 2000; Safi and Kerth, 2004) by creating a dependence on very specific resources (Begon et al., 1996a; Begon et al., 1996b). Thus, to contribute to the conservation of *R. hipposideros* it is important to understand if this species has a specialized diet with a narrow spectrum, or if is a generalist species that feeds on a large spectrum of arthropod types (Begon et al., 1996b; Drickamer et al., 1996) likely to be found even on modified habitats.

In this study we analyzed the diet of one of the largest nursing colony of *R. hipposideros* in Portugal. Specifically, we aimed to a) characterize the diet of *R. hipposideros* in this modified habitat; b) characterize the abundance and diversity of potential prey in the area; and c) compare diet with food availability to test for active prey selection. Finally, we compared the dietary habits of *R. hipposideros* in this modified Mediterranean habitat with those of Central and Western European populations.

## 4.3. Materials and Methods

### 4.3.1. Study Area

From February to October 2012, we studied the seasonal variation in the composition and abundance of insects in the diet of *R. hipposideros* from one of the largest colony known in Portugal. The roost, occupied since 2002, is an ancient human infrastructure located in

Sintra (Fig. 4.1), and has been consistently monitored under the initiative of the Sintra Speleological Group (AES). The colony is composed of around 150 individuals most of them are females and their newborns, and a small portion are males.

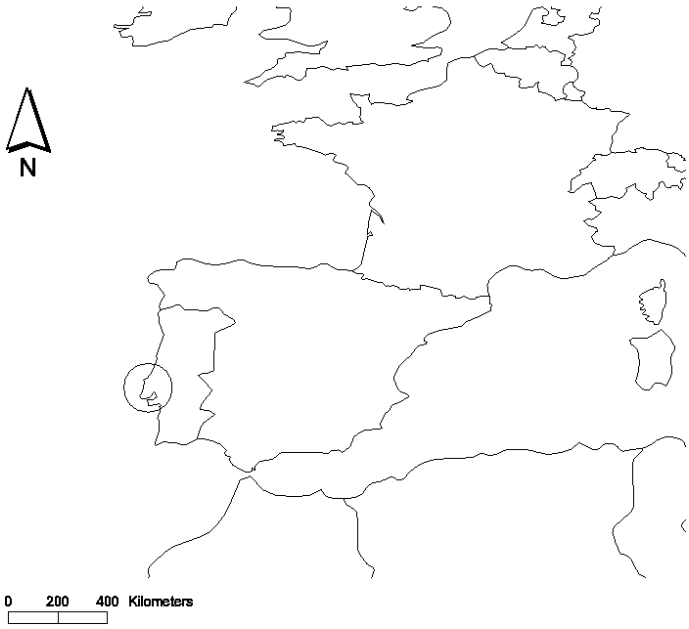



Fig. 4.1 Approximate location of the study area (Sintra, Portugal).

The Sintra mountain range presents a very specific microclimate within the Mediterranean region, with high relative humidity levels, mildly cold temperatures (average: 14.8 °C/year) and significant levels of rainfall for a Mediterranean region that occurs mostly during winter (average: 1006 mm/year; Coutinho et al., 2012; Lousã et al., 2005). The landscape of Sintra, and in particular the area surrounding the roost, is characterized by a typically Mediterranean vegetation composed of some patches of *Quercus* spp. and many trees and shrubs of *Pinus pinea*, *Ulex* sp. and *Ilex aquifolium*, interlaced with small agricultural fields as well as some plantations of *Pinus pinaster* and *Cupressus lusitanica*. However, a large area is characterized by exotic vegetation, such as *Eucalyptus globulus*,

*Acacia* spp., *Pittosporum undullatum*, *Hakea salicifolia*, *Hakea sericea* and *Aillanthus altissima*.

### 4.3.2. Diet and food availability

The analysis of bat droppings presents some practical limitations, but several works show that faecal analysis can yield reasonable estimates of the food ingested by insectivorous bats (Kunz and Whitaker, 1983). We collected faecal pellets of *R. hipposideros* by placing a cloth sheet beneath the colony for a period of four days in each month between February to October 2012 since, during the colder months of November, December and January, the roost is seldom used. A random selection of 20 pellets was taken in each month, so a total of 180 pellets were analysed. The pellets were left to soak in 70% ethanol for at least 4 hours. Individual pellets were then placed in a Petri dish and a few drops of glycerine were added to aid the separation of the fragments contained in the pellets. The remains of prey were inspected using a binocular microscope (Shiel et al., 1997). Whenever possible, the fragments were identified to the family level. However, because the Rhinolophidae are able to degrade chitin due to the presence of chitinase in their digestive tract (Jeuniaux, 1961), in several cases we were only able to identify the food items to the order level. Item identification was made using a reference collection of arthropods collected in the area and arthropod identification guides (Shiel et al., 1997; Whitaker, 1988). The families Ceratopogonidae and Chironomidae are difficult to distinguish in pellets so we grouped these two families. Because Lepidoptera scales remain in the digestive tract of bats for quite a long time, we only considered the presence of this family when other remnants were also present in the bat droppings, *e.g*. legs and proboscis (Arlettaz et al., 2000; Kunz and Whitaker, 1983).

The abundance of potential food resources was sampled at the same time as the pellets, during the same four days per month, using a light trap placed in the proximity of the roost (ca. 100 m). The arthropods collected were preserved in 70% ethanol. As the prey size of *R. hipposideros* is between 3 and 14 mm (Blanco, 1998), only arthropods within this range were selected for identification. Arthropods were identified to family level or, when this

was not possible, to order level using identification guides (Baez, 1988; Chinery, 2007; Galante-Patiño and Viejo, 1988; Gayudo, 1988; Goulet et al., 1993; Martinez, 1988; Mier, 1988; Salgado et al., 1988; Unwin, 1988; Vazquez, 1988; Waring and Townsend, 2009).

### 4.3.3. Statistical analysis

The relative importance of each prey type was calculated as the percentage frequency in the droppings and in availability. Monthly comparisons of diet and food availability for each taxa were done by comparing the occurrence of arthropods found in the pellets, i.e. the proportion of the pellets where the prey occurred, with the relative and absolute abundance of the same taxon in terms of availability (Shiel et al., 1997).

Significant monthly variation in diet and prey availability was tested using the Pearson Chi-squared test (Zar, 1999). The Shannon-Wiener diversity index (Krebs, 1989) was used to analyse the heterogeneity in the diet and in the availability of prey.

Prey selection was investigated using logistic regression models (Hosmer and Lemeshow, 1989). We followed the approach of Ramos Pereira et al. (2002) where the presence/absence of a prey type in the pellets is the dependent variable, and the predictor variables are the absolute and relative abundance of each prey type in terms of availability. In the absence of prey selection, the most abundant prey in the diet will be the one with greatest relative abundance. In the case of prey selection, the probability of the presence of the preferred prey in the diet will depend on its absolute abundance, rather than on the relative abundance. In that case, the probability of the presence of the other prey taxa will be negatively influenced by the absolute abundance of the preferred taxon. Analyses were done in R version 3.0.1 (R Core Team 2013).

## 4.4. Results

### 4.4.1. Diet and food availability

The diet of the studied population of *R. hipposideros* was dominated by Lepidoptera (19.7%), Tipulidae (Diptera, 19.1%), Hymenoptera (16.6%), other Diptera (13.9%) and Neuroptera (11.7%). Ceratopogonidae/Chironomidae (Diptera) was found with percentage frequencies of 4.7%. Ptinidae (Coleoptera) and Cecidomyiidae were found in 2.9% of the pellets. Other taxa with lower relevance (<2%) included Psychodiidae (Diptera), other Coleoptera, Araneae, Hemiptera and Mycethophilidae (Diptera).

We caught 2079 arthropods with sizes between 3 and 14 mm using the light trap. The most represented taxa were: Ceratopogonidae/Chironomidae (Diptera, 52.5%), Coleoptera (21.2%), Diptera (8.0%), Lepidoptera and Hymenoptera (5.2%). Other less represented taxa (<5%) included Hemiptera, Psocoptera, Cecidomyiidae (Diptera), Mycetophilidae (Diptera), Psychodidae (Diptera), Tipulidae (Diptera), Araneae and Ptinidae (Fig. 4.2).

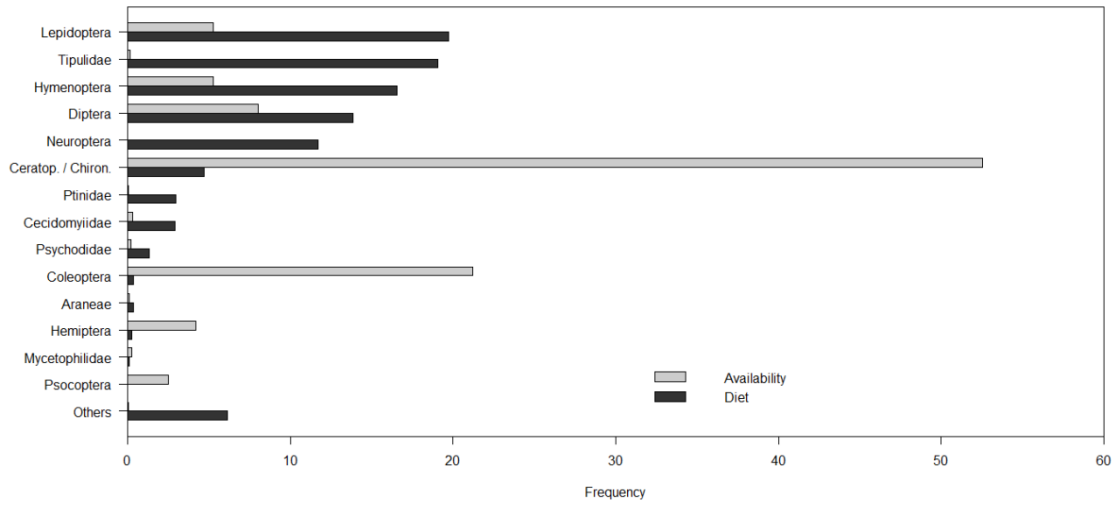



Fig. 4.2 Percentage frequency of prey found in faecal pellets of *R. hipposideros* (dark grey) and of arthropods captured in the light traps (light grey).

### 4.4.2. Monthly variation

The diet of *R. hipposideros* did not show significant variation among the months of study ($\chi 2 = 218.24$, d.f. = 32, p-value = 0.9755). Indeed, the frequency of Lepidoptera, Tipulidae, Hymenoptera and other Diptera in the diet was relatively constant throughout the sampling period (Fig. 4.3).

However, the availability of prey showed significant monthly variation ($\chi 2 = 350.3$, d.f. = 24, p-value = 2.2e-16), with Ceratopogonidae/Chironomidae, Coleoptera, Diptera and Lepidoptera showing strong variation across months (Fig. 4.3).

The Shannon-Wiener diversity index indicated that dietary diversity was relatively constant, with only a slight reduction in June. The diversity of available prey was higher in April, July and August, and very low in February, September and October (Fig. 4.3).

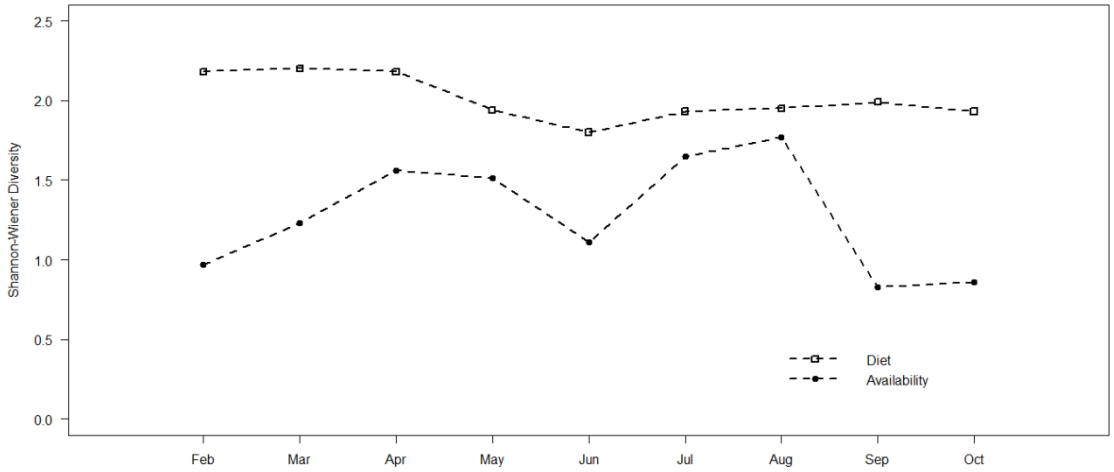
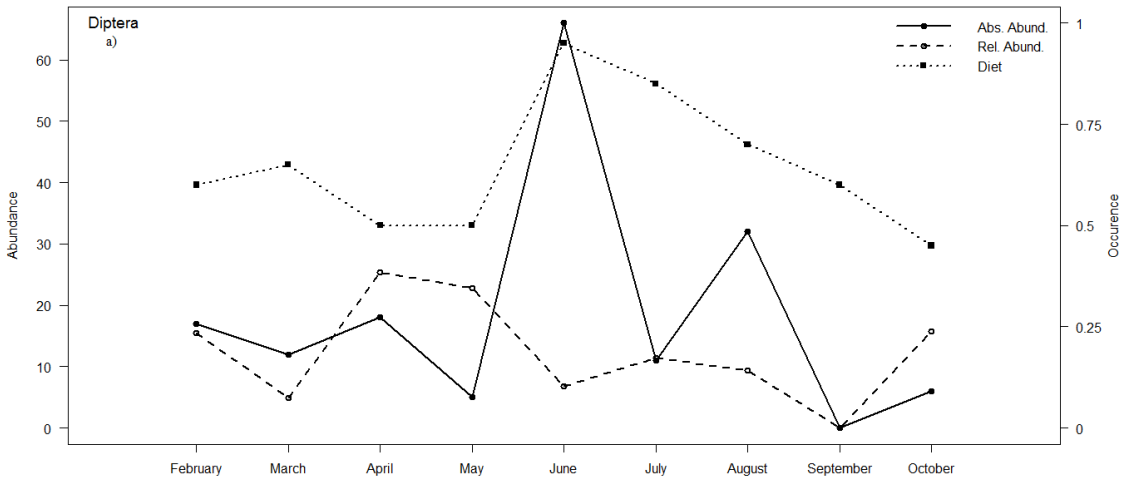



Fig. 4.3 Shannon-Wiener diversity index per sampling month.

### 4.4.3. Prey selection

Diptera seemed to be the only taxa preferentially selected by *R. hipposideros* in the study. There appeared to be a relation between the presence of Diptera in the diet and its absolute abundance in terms of availability (Fig. 4.4). The results of the logistic regression models, using the occurrence of Diptera in the diet as the response variable and the absolute abundance of Diptera as the predictor variable, indicated that Diptera were indeed being selected (Table 4.1). The occurrence of Hymenoptera and Lepidoptera in the diet was relatively constant and did not follow the peaks of absolute abundance of those taxa in terms of availability (Fig. 4.4), which is corroborated by the results of the logistic regression models (Table 4.1). Seasonality did not seem to influence the occurrence of any of these three main prey items in the diet (Table 4.1).



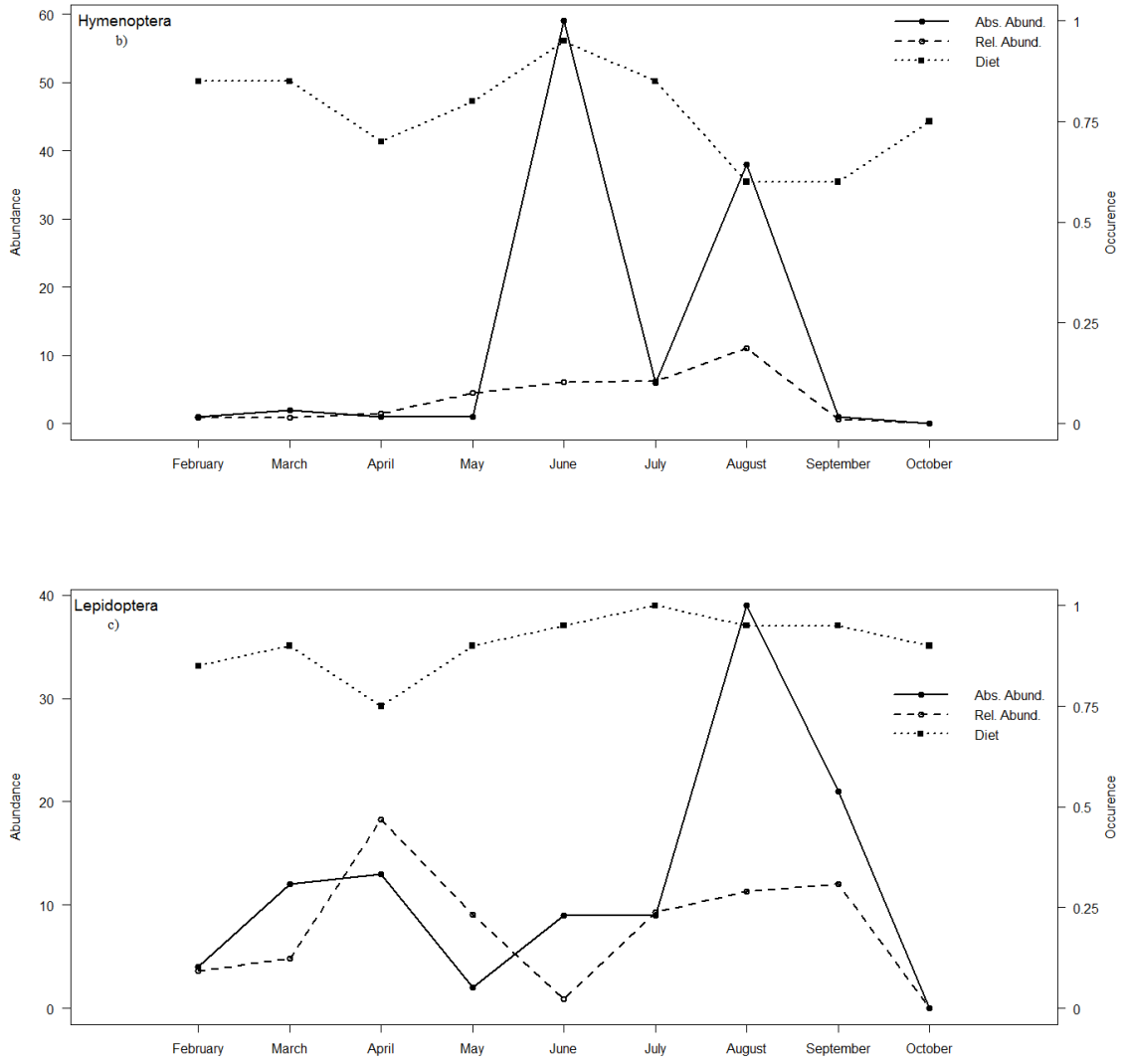



Fig. 4.4 Monthly variation of absolute abundance and relative abundance in the availability and occurrence in the diet of: a) Diptera, b) Hymenoptera and c) Lepidoptera.

Table 4.1 Results of the fitted logistic regression models using absolute abundance of prey and month as predictor variables for the presence of each of the main taxa in the diet.

| Taxon | p-value | | d.f. |
|---|---|---|---|
| | Absolute Abundance | Months | |
| Diptera | 0.046 | 0.802 | 2 |
| Hymenoptera | 0.411 | 0.161 | 2 |
| Lepidoptera | 0.801 | 0.203 | 2 |

To try to better understand the importance of these two taxa in the diet when the abundance of Diptera decreases, new models were built using the occurrence of Hymenoptera and Lepidoptera in the diet as the response variables and the absolute abundance of Diptera and the relative abundance of Hymenoptera and Lepidoptera as the predictor variables. None of these models were statistically significant (Table 4.2), which suggests that even when Diptera are scarcer, no other taxon seems to be actively chosen. However, it should be noted that Neuroptera and Tipulidae are two important taxa in the diet of *R. hipposideros,* but are practically non-existent in terms of availability (Fig. 4.5), which prevented any logistic regression models from being elaborated to statistically test if these two taxa were indeed actively selected.

Table 4.2 Results of logistic regression models using absolute abundance of Diptera and relative abundance of Hymenoptera and Lepidoptera as predictor variables for testing the importance of these two taxa in the diet when the abundance of Diptera decreases.

| Taxon | p-value | | d.f. |
|---|---|---|---|
| | Absolute Abundance (Diptera) | Relative abundance (Hymenopter and Lepidoptera) | |
| Hymenoptera | 0.170 | 0.354 | 2 |
| Lepidoptera | 0.929 | 0.439 | 2 |

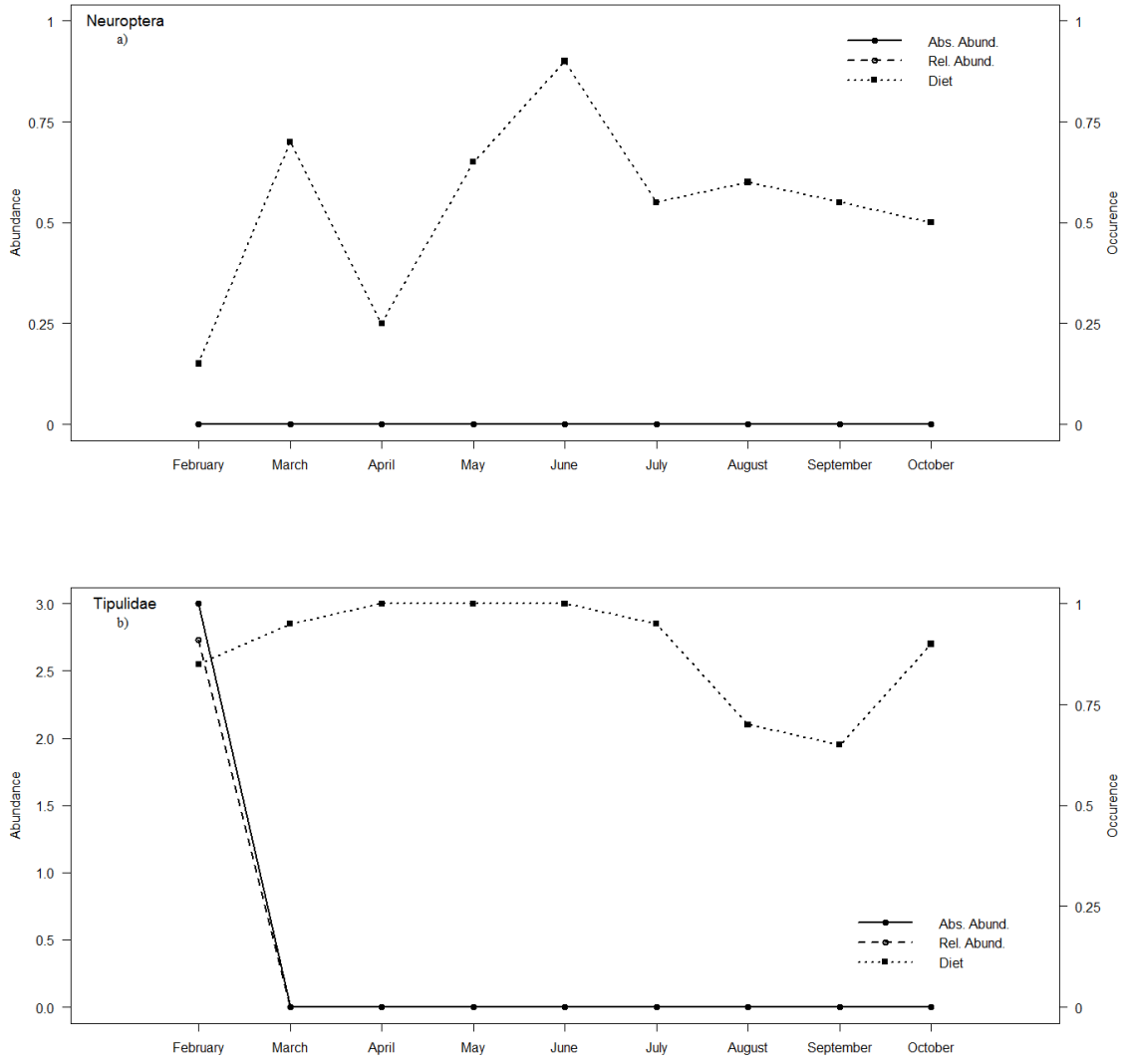



Fig. 4.5 Monthly variation in the occurrence of a) Neuroptera and b) Tipulidae in the diet of R. hipposideros, and their absolute and relative abundance in terms of availability.

## 4.5. Discussion

This study demonstrates that, in our study area, the diet of *R. hipposideros* is essentially composed of Lepidoptera, Tipulidae, Hymenoptera, Diptera and Neuroptera. These results are identical to those of studies done in Western and Central Europe (Arlettaz et al., 2000; McAney and Fairley, 1989), suggesting that *R. hipposideros* feeds on similar taxa all across Europe, even though our study was done in a significantly human-altered habitat.

However, to our knowledge, this is the first study in which Hymenoptera has a fairly important representation in the diet of *R. hipposideros*.

Prey occurrence in the diet did not follow the same patterns in terms of availability. The prey items in the diet of *R. hipposideros* remain relatively constant, while food availability clearly shows seasonal variation. This suggests that *R. hipposideros* does not feed according to the abundance of prey available in the environment. While feeding on a great number of prey items, and thus showing a broad dietary niche, it may be considered a generalist species (Begon et al., 1996b; Drickamer et al., 1996). Nonetheless, though a generalist species it seems to have preference for some dietary items. In fact, the logistic regression models show Diptera as an actively selected prey, since its occurrence in the diet of *R. hipposideros* follows its pattern in terms of absolute abundance. This is consistent with the work of Vaughan (1997) who demonstrated that, in Britain, this taxon is the most important in the diet of *R. hipposideros*, even during the winter (Williams et al., 2011).

The presence of some preferential food items is not surprising because other rhinolophids also seem to show a clear preference by certain arthropod orders. Indeed, both *Rhinolophus euryale* and *Rhinolophus mehelyi* show a preference for moths in the Iberian Peninsula (Goiti et al., 2004). While apparently not actively selecting Lepidoptera and Hymenoptera, these are also important components of the diet of *R. hipposideros*. Again, this argues for its generalist feeding behaviour but not necessarily an opportunistic one. Indeed, while Chironomidae and Ceratopogonidae are highly available, they seldom occur in the diet of *R. hipposideros*, somehow supporting the idea that this species does not feed according to the availability of potential prey. These two taxa harbour some of the smallest insects within the size range of *R. hipposideros* dietary items, suggesting that it prefers slightly larger prey, possibly because they are more cost effective, which is in line with the optimal foraging theory (MacArthur and Pianka, 1966).

Coleoptera is a frequent taxon in the surroundings of the roost but uncommon in the diet of *R. hipposideros* in the studied colony, supporting the belief that these bats mainly eat soft-bodied arthropods (Arlettaz et al., 2000; Williams et al., 2011). It should also be noted that Tipulidae and Neuroptera were underrepresented in the sampling of prey availability, despite occurring frequently in the diet of *R. hipposideros*. There are two possible explanations for this. First, the sampling method used for prey availability may not have

been the most appropriate to capture these two orders. However, other authors have used light trapping techniques to actively capture Tipulidae and Neuroptera (Andersen and Greve, 1975; Kimura et al., 2011). So, an alternative hypothesis is that these two orders are indeed rare in the study area, but *R. hipposideros* actively searches for them. In fact, in Ireland, McAney and Fairley (1989) also found a high frequency of Neuroptera in the diet of *R. hipposideros*, but never captured this taxon in their traps.

Studies with Rhinolophidae have shown that these bats can discriminate certain characteristics of their prey and hence are capable of selective feeding (Goiti et al., 2004; Jones, 1990; Ma et al., 2008; Salsamendi et al., 2008), which supports our theory of Diptera selection by *R. hipposideros*. By having a sophisticated system of echolocation, the Rhinolophidae are able to detect prey even when those prey items are hidden in the vegetation, provided that they are flying or flapping their wings (Ma et al., 2008). Actually, *R. hipposideros*, which essentially feeds within the clutter of woodlands (Schofield, 1996), is capable of differentiating the characteristics of returning echoes produced by foliage and by prey. The pure tone signal of constant frequency signals from foliage, for example, maintains its unaltered structure even when foliage flutters due to wind (Jones and Teeling, 2006; Neuweiler, 1989), but when a fluttering target appears, the pure tone echo will carry distinct glints that create "echo signatures" (Neuweiler, 1989; Von der Emde and Menne, 1989). Each wing beat is distinctly coded by horseshoe bats, so that they can use echolocation for detecting and also for identifying specific wing beat patterns by analysing the spectrum of their echoes (Neuweiler, 1989). This, together with the capacity to explore Doppler-shift echoes, makes the echolocation of horseshoe bats the most adequate for searching for moving prey in cluttered habitats and renders them extremely efficient in classifying their targets (Jones and Teeling, 2006). This probably allows them to be more selective in terms of prey, as occurs in our study in Sintra.

It should be underlined that faecal analysis may be somewhat biased but, together with molecular analyses, it is the most ethical option. Certainly, the application of molecular studies to the analysis of the diet of bats allows a greater resolution of prey identity than that possible through the direct examination of faecal material (Zeale et al., 2011), but it is much more expensive. Also, in bat dietary studies using visual faecal analysis, the overrepresentation of arthropods with hard-bodies when compared with insects with soft-bodies seems to be significant (Dickman and Huang, 1988; Kunz and Whitaker, 1983).

However, this problem does not seem to be an issue with *R. hipposideros* because this species appears to prefer soft-bodied insects (Williams et al., 2011).

The coincidence in dietary composition among different regions and the diversity of items consumed by *R. hipposideros* in this modified forest in Southwest Europe – as occurs in pristine environments and other human-altered areas in Europe (Arlettaz et al., 2000; McAney and Fairley, 1989) – suggests that habitats that maintain high insect diversity are required by this species for foraging. Indeed, *R. hipposideros* is known to select broadleaved woodlands and riparian vegetation (Bontadina et al., 2008; Bontadina et al., 2002; Reiter, 2004a), where insects seem to be more diverse and abundant (Goiti et al., 2004), over conifer plantations, settlements and open areas. So, even in highly modified landscapes, it is likely that *R. hipposideros* searches for patches of native forests to forage, making the protection of these areas and the maintenance of forested corridors among them crucial for the conservation of this species.

# Chapter 5

General discussion

# 5. General discussion

In this work we described daily and monthly patterns of use of a maternity roost by *Rhinolophus hipposideros* and evaluated how external climatic factors influence the nocturnal activity. We also characterized the diet of that maternity colony between February and October and, by evaluating the availability of potential prey in the surroundings of the roost, we also tested for the existence of active prey selection by these bats.

## 5.1. Occupation of the roost

The peak of occupation of the studied roost occurred during the breeding season. The choice of the adequate roost for each phase of the life-cycle of bats is extremely important in Holarctic bats. Indeed, because climatic conditions change significantly along the year, choosing roosts with relatively constant ambient conditions or that present a range of microclimates reduces the costs with thermoregulation, which is especially relevant during hibernation and breeding.

Winter represents a critical energy bottleneck for small bats in temperate regions (Turbill and Geiser, 2008), but the breeding season is the phase of the life-cycle of bats that is energetically more costly (Racey and Entwistle, 2000). In the studied roost, temperature is controlled by artificially heating of some areas of the roost. We believe that the increased use of the roost in the last few years is due to this availability of relatively different (but warm), microclimates across the roost, creating suitable conditions for bats along the different periods of the breeding season, in particular the development of gestation, milk production and postnatal growth (Dietz et al., 2007; Herreid, 1967; Kerth et al., 2001; Rodrigues and Palmeirim, 2008), even if the adult size of bats is also obviously correlated with nutritional input (Dietz et al., 2007).

### 5.2. Time of births

During the temperate winter foraging is, for the majority of time, unproductive because arthropod availability is very low (Williams, 1939; Williams, 1961) and so bats enter a period of hibernation (Speakman and Thomas, 2003). On the other hand, parturition, lactation and weaning usually coincide with the maximum food availability, and this is true for both temperate (Arlettaz et al., 2001; Frick et al., 2010) and tropical and subtropical bats (Ramos Pereira et al., 2010). *R. hipposideros* is no exception: in the studied colony, parturition occurs in late May/early June, sooner than in higher latitudes (Reiter, 2004b; Schofield, 1996). In fact, the absolute abundance of most of the prey items consumed by *R. hipposideros* is maximum is June, with the exception of Lepidoptera that shows the higher peak of abundance in August. So, in temperate bats, selection has favoured parturition in late spring or early summer because: i) the high-energy costs of lactation coincide with the greatest seasonal resource availability (Arlettaz et al., 2001; Bronson, 1985; Racey and Entwistle, 2000), certainly evident in the studied colony of *R. hipposideros*; and ii) it gives young bats enough time to learn to fly and forage independently, and to reserve enough fat stores to survive across the hibernation period (Kunz et al., 1998).

### 5.3. Activity patterns

Foraging activity patterns vary between species and, within the same species, between sites. In fact, the activity pattern of *R. hipposideros* in our study differs from that described by McAney and Fairley (1988) in Ireland. Changes in nocturnal activity patterns throughout the year are also frequent in bats. For example, *Pipistrellus pipistrellus* shows a unimodal pattern of activity during pregnancy that becomes bimodal during lactation (Swift, 1980). This change in behaviour is certainly determined by energetic constraints. Flight is energetically costly (Winter and Von Helversen, 1998) and energy expenditure increases towards the end of pregnancy when wing-loading is higher (Jones, 1990). So, one unique flight during pregnancy can be economically more advantageous than two flights

during the night (Swift, 1980). However, this pattern alters when females give birth and must return at least once to the roost during the night to suckle their young (Maier, 1992; Swift, 1980).

The patterns in *R. hipposideros* are not that straightforward: in Ireland, *R. hipposideros* presents intermittent activity throughout the night, with many individuals returning and departing, with no evident seasonal or overnight peaks (McAney and Fairley, 1988) while in our colony the foraging activity of *R. hipposideros* was bimodal throughout all summer months. Nevertheless, in both areas the activity of *R. hipposideros* seems to be dictated by light intensity, which is not surprising since it feeds on crepuscular insects that have swarming behaviour (Knight and Jones, 2009).

The foraging activity of *R. hipposideros* is also influenced by other factors such as temperature, wind speed and precipitation. As shown for other insectivorous bats, e.g. *Myotis lucifugus*, *Chalinolobus tuberculatus* and *Hypsugo savii* (Anthony et al., 1981; Griffiths, 2007; O'Donnell, 2000; Russo and Jones, 2003), the activity of *R. hipposideros* in Sintra also is affected by temperature. In fact, the negative influence of low temperatures in bat activity may be correlated with the reduction of food availability. Low insect densities probably prevent significant energetic gains, so bats spend more time in the roost (Anthony et al., 1981; Ciechanowski et al., 2007; O'Donnell, 2000). The activity of flying insects can also be negatively affected by wind making them unavailable for bats (Peng et al., 1992) while simultaneously increasing the energy required for bats to fly. Really, in a study realised in Portugal, Amorim et al. (2012) found that activity of *Nyctalus leisleri* and *P. pipistrellus* is higher with wind speed lower than 5.0 $m.s^{-1}$ and for high levels of wind, the activity of these two species is reduced.

In Ireland rain seems to negatively affect the activity of the lesser horseshoe bat (McAney and Fairley, 1988) and this also occurred in Sintra. However, this pattern is not similar for every species. For instance, *Eptesicus serotinus* and *Chalinolobus tuberculatus*, continue to be active under rain (Catto et al., 1995; Griffiths, 2007) while in *E. serotinus*, *R. hipposideros* and *Macrophyllum macrophyllum*, heavy rain may delay bat emergence or even completely inhibit activity (Catto et al., 1996; McAney and Fairley, 1988; Weinbeer et al., 2006). The reduction of flight activity during rain periods can be explained by energetic costs, because rain seems to significantly increase energy expenditure during flight. Indeed, Voigt et al. (2011), found that flight metabolism of *Carollia sowelli* doubled

when bats were wet or when they were exposed to rain. Weinbeer et al. (2006) also suggest that the reduction of activity of *M. macrophyllum* under rain can be explained by the difficulty of this species to discriminate between rain droplets and the aerial insects it preys.

### 5.4. Diet

*R. hipposideros* is known to catch its prey by hawking, gleaning and in late pregnancy using flycatcher-style (Jones and Rayner, 1989; Schofield, 1996). At least at the order and family level, the diet of *R. hipposideros* does not seem differ substantially between the different regions (e.g. Ireland, Switzerland and Portugal) as the main consumed taxa were consistently Lepidoptera, Diptera and Neuroptera (Arlettaz et al., 2000; McAney and Fairley, 1989). Still, some taxa are replaced by others in different regions: for instance, in Ireland, Trichoptera constitutes 18% of the diet (McAney and Fairley, 1989) while in Portugal, this taxon seems be replaced by Hymenoptera, where it constitutes 17% of the diet (this study).

Schoener (1971) defines a generalist species as that foraging on a large range of food types. Where food availability is affected differentially and unpredictably, generalist feeding is usually favoured. This occurs more frequently in temperate species, such as *R. hipposideros*.

On the other hand, the optimal foraging theory hypothesizes that each animal selects food in terms of their cost and benefit, i.e. each potential food item has a intrinsic value based on: i) its nutrient and energy content, ii) how much time is required to extract the food (handling time), and iii) how long it takes to locate the food (search time) (Yahnke, 2006). *R. hipposideros* feeds on many crepuscular insects that present swarming behaviour, reducing handling and searching time. However, under this theory we would expect a greater consumption of Ceratopogonidae and Chironomidae, due to their high availability. The possible explanation for this pattern is the fact that these two families present the smallest size within the range of items consumed by *R. hipposideros* making them less profitable in terms of energy gain. This is probably more evident when other larger prey

are available – within the range size of items consumed by *R. hipposideros* – as seems to be the case of other families of Diptera actively selected by *R. hipposideros*.

### 5.5. Future work

During the last months of 2012 additional equipment was set up in the "Quinta da Regaleira" roost with the purpose of improving the ongoing bat monitoring. Four more cameras and data loggers are now monitoring the internal ambient parameters of the roost. A meteorological station was also set outside the roost. Because this new equipment only started to run regularly from the beginning of 2013 we were not able to use its data in this work.

One important aspect that was left behind was the study of the relation between the internal environmental conditions of the roost and its use by the bats. We only had sporadic measurements of those parameters, and because the roost was heated we assumed that, at least in terms of temperature, the conditions inside the roost were more or less constant. Relating the occupation of different sites of the roost with the microclimate of each site will certainly bring additional information about the best environmental conditions for different phases of the life-cycle of *R. hipposideros*, supporting future developments of artificial roosts.

The management and conservation of foraging areas is essential for the conservation of *R. hipposideros*. So, it would be important to identify the foraging habitats of this colony using radio-tracking or even light tags coupled with night scopes, as this species does not seem to travel far to forage. This information would also serve as a starting point for habitat restoration, aiming to increase the availability of high-quality foraging habitats for *R. hipposideros* in the region.

# Chapter 6

References

# 6. References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# Chapter 7

Appendix

# 7. Appendix

Table 7.1 Monthly mean, maximum and standard deviation of individuals that occupied the roost during 2010. M.m. – Monthly mean; Max. – Maximum; St.dev. – Standard deviation.

| | July | | | August | | | October | | |
|---|---|---|---|---|---|---|---|---|---|
| Hour (hh:mm) | M.m. | Max. | St.dev | M.m. | Max. | St.dev | M.m. | Max. | St.dev |
| 00:00 | 38 | 86 | 26 | 19 | 42 | 13 | 118 | 148 | 21 |
| 01:00 | 84 | 155 | 46 | 58 | 98 | 32 | 116 | 153 | 24 |
| 02:00 | 110 | 179 | 47 | 88 | 133 | 47 | 110 | 145 | 26 |
| 03:00 | 110 | 167 | 39 | 92 | 139 | 50 | 105 | 147 | 28 |
| 04:00 | 106 | 150 | 38 | 92 | 141 | 48 | 98 | 133 | 26 |
| 05:00 | 72 | 103 | 29 | 80 | 127 | 41 | 95 | 126 | 26 |
| 06:00 | 40 | 67 | 21 | 27 | 47 | 14 | 92 | 127 | 22 |
| 07:00 | 69 | 112 | 37 | 59 | 89 | 29 | 36 | 52 | 11 |
| 08:00 | 75 | 115 | 38 | 72 | 101 | 35 | 1 | 5 | 1 |
| 09:00 | 81 | 119 | 37 | 78 | 105 | 38 | 1 | 5 | 1 |
| 10:00 | 87 | 129 | 33 | 83 | 107 | 38 | 1 | 4 | 1 |
| 11:00 | 93 | 131 | 29 | 86 | 108 | 36 | 1 | 4 | 1 |
| 12:00 | 96 | 133 | 26 | 87 | 107 | 33 | 1 | 4 | 1 |
| 13:00 | 96 | 132 | 25 | 88 | 109 | 32 | 1 | 4 | 1 |
| 14:00 | 91 | 132 | 27 | 88 | 109 | 34 | 1 | 4 | 1 |
| 15:00 | 90 | 133 | 28 | 89 | 110 | 33 | 1 | 4 | 1 |
| 16:00 | 88 | 133 | 29 | 86 | 107 | 33 | 1 | 4 | 1 |
| 17:00 | 81 | 133 | 35 | 85 | 105 | 31 | 1 | 4 | 1 |
| 18:00 | 74 | 110 | 27 | 88 | 107 | 31 | 0 | 2 | 1 |
| 19:00 | 75 | 113 | 28 | 84 | 107 | 30 | 2 | 6 | 2 |
| 20:00 | 67 | 115 | 33 | 63 | 89 | 26 | 33 | 50 | 11 |
| 21:00 | 28 | 52 | 18 | 13 | 22 | 8 | 77 | 100 | 22 |
| 22:00 | 23 | 35 | 12 | 30 | 59 | 16 | 103 | 136 | 26 |
| 23:00 | 33 | 55 | 20 | 28 | 43 | 12 | 114 | 142 | 25 |

Table 7.2 Monthly mean, maximum and standard deviation of individuals that occupied the roost during March, April and May 2012. M.m. – Monthly mean; Max. – Maximum; St.dev. – Standard deviation.

| | March | | | April | | | May | | |
|---|---|---|---|---|---|---|---|---|---|
| Hour (hh:mm) | M.m. | Max. | St.dev | M.m. | Max. | St.dev | M.m. | Max. | St.dev |
| 00:00 | 17 | 28 | 9 | 20 | 38 | 10 | 20 | 48 | 13 |
| 01:00 | 20 | 34 | 9 | 20 | 43 | 11 | 26 | 56 | 13 |
| 02:00 | 23 | 31 | 7 | 19 | 38 | 10 | 27 | 49 | 14 |
| 03:00 | 23 | 26 | 3 | 17 | 32 | 8 | 26 | 50 | 14 |
| 04:00 | 25 | 29 | 5 | 15 | 28 | 7 | 26 | 59 | 14 |
| 05:00 | 29 | 38 | 6 | 15 | 39 | 8 | 20 | 39 | 11 |
| 06:00 | 24 | 30 | 6 | 10 | 25 | 6 | 20 | 56 | 16 |
| 07:00 | 4 | 6 | 2 | 12 | 26 | 9 | 28 | 69 | 19 |
| 08:00 | 6 | 9 | 4 | 12 | 26 | 8 | 29 | 71 | 19 |
| 09:00 | 7 | 10 | 4 | 11 | 24 | 7 | 29 | 72 | 19 |
| 10:00 | 6 | 11 | 5 | 11 | 24 | 8 | 29 | 74 | 19 |
| 11:00 | 6 | 11 | 5 | 11 | 25 | 8 | 30 | 76 | 19 |
| 12:00 | 7 | 12 | 5 | 12 | 27 | 8 | 30 | 77 | 18 |
| 13:00 | 6 | 10 | 5 | 12 | 27 | 8 | 30 | 75 | 18 |
| 14:00 | 6 | 10 | 4 | 12 | 24 | 8 | 30 | 72 | 18 |
| 15:00 | 6 | 10 | 4 | 12 | 27 | 8 | 29 | 61 | 18 |
| 16:00 | 6 | 10 | 4 | 11 | 26 | 8 | 30 | 58 | 17 |
| 17:00 | 6 | 10 | 4 | 11 | 28 | 8 | 30 | 58 | 16 |
| 18:00 | 5 | 8 | 4 | 11 | 28 | 8 | 30 | 60 | 16 |
| 19:00 | 4 | 9 | 4 | 11 | 25 | 8 | 28 | 60 | 16 |
| 20:00 | 2 | 4 | 2 | 5 | 15 | 4 | 19 | 49 | 13 |
| 21:00 | 5 | 9 | 3 | 4 | 13 | 3 | 5 | 13 | 4 |
| 22:00 | 12 | 13 | 1 | 12 | 27 | 5 | 15 | 39 | 11 |
| 23:00 | 16 | 21 | 4 | 17 | 32 | 8 | 19 | 36 | 10 |

Table 7.3 Monthly mean, maximum and standard deviation of individuals that occupied the roost during June, July, August and October 2012. M.m. – Monthly mean; Max. – Maximum; St.dev. – Standard deviation.

| | June | | July | | | August | | | October | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Hour (hh:mm) | M.m. | St.dev | M.m. | Max. | St.dev | M.m. | Max. | St.dev | M.m. | Max. | St.dev |
| 00:00 | 37 | 0 | 46 | 62 | 15 | 14 | 27 | 9 | 59 | 91 | 25 |
| 01:00 | 42 | 0 | 84 | 96 | 12 | 54 | 62 | 7 | 62 | 81 | 23 |
| 02:00 | 41 | 0 | 95 | 104 | 8 | 92 | 101 | 10 | 60 | 90 | 29 |
| 03:00 | 30 | 0 | 92 | 105 | 12 | 98 | 109 | 6 | 60 | 91 | 31 |
| 04:00 | 31 | 0 | 88 | 96 | 7 | 93 | 103 | 10 | 56 | 85 | 31 |
| 05:00 | 18 | 0 | 40 | 46 | 6 | 71 | 104 | 24 | 56 | 92 | 35 |
| 06:00 | 54 | 0 | 70 | 74 | 8 | 44 | 70 | 18 | 41 | 70 | 26 |
| 07:00 | 62 | 0 | 106 | 117 | 15 | 110 | 135 | 23 | 10 | 20 | 8 |
| 08:00 | 61 | 0 | 100 | 108 | 11 | 112 | 137 | 24 | 8 | 17 | 7 |
| 09:00 | 60 | 0 | 101 | 110 | 15 | 117 | 156 | 29 | 9 | 19 | 9 |
| 10:00 | 58 | 0 | 98 | 114 | 20 | 116 | 151 | 29 | 9 | 19 | 9 |
| 11:00 | 58 | 0 | 104 | 121 | 17 | 117 | 149 | 28 | 9 | 19 | 9 |
| 12:00 | 58 | 0 | 102 | 116 | 13 | 114 | 144 | 28 | 7 | 16 | 8 |
| 13:00 | 60 | 0 | 94 | 103 | 9 | 113 | 136 | 23 | 8 | 17 | 8 |
| 14:00 | 63 | 0 | 99 | 113 | 14 | 106 | 129 | 22 | 7 | 15 | 7 |
| 15:00 | 59 | 0 | 94 | 103 | 9 | 103 | 129 | 23 | 6 | 15 | 6 |
| 16:00 | 61 | 0 | 96 | 107 | 15 | 98 | 131 | 25 | 6 | 14 | 6 |
| 17:00 | 60 | 0 | 98 | 109 | 18 | 102 | 126 | 20 | 6 | 14 | 6 |
| 18:00 | 56 | 0 | 95 | 113 | 20 | 106 | 128 | 18 | 7 | 13 | 6 |
| 19:00 | 57 | 0 | 103 | 115 | 17 | 112 | 132 | 13 | 8 | 18 | 6 |
| 20:00 | 47 | 0 | 101 | 105 | 6 | 63 | 85 | 22 | 25 | 39 | 14 |
| 21:00 | 11 | 0 | 20 | 28 | 7 | 13 | 22 | 8 | 27 | 39 | 12 |
| 22:00 | 36 | 0 | 36 | 46 | 11 | 42 | 82 | 21 | 12 | 23 | 7 |
| 23:00 | 26 | 0 | 32 | 36 | 6 | 21 | 49 | 16 | 14 | 21 | 5 |

Table 7.4 Monthly mean, maximum and standard deviation of juveniles that occupied the roost during July and August from 2010 and 2012. M.m. – Monthly mean; Max. – Maximum; St.dev. – Standard deviation.

| | 2010 | | | | | | 2012 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | July | | | August | | | July | | | August | | |
| Hour (hh:mm) | M.m. | Max. | St.dev | M.m. | Max. | St.dev | M.m. | Max. | St.dev | M.m. | Max. | St.dev |
| 00:00 | 4 | 8 | 2 | 2 | 2 | 0 | 9 | 12 | 4 | 1 | 2 | 1 |
| 01:00 | 7 | 14 | 4 | 4 | 9 | 3 | 13 | 18 | 4 | 2 | 4 | 2 |
| 02:00 | 11 | 23 | 8 | 6 | 9 | 3 | 16 | 22 | 6 | 4 | 8 | 3 |
| 03:00 | 13 | 24 | 8 | 6 | 9 | 2 | 13 | 19 | 5 | 7 | 11 | 4 |
| 04:00 | 14 | 26 | 9 | 6 | 12 | 3 | 11 | 14 | 2 | 6 | 11 | 4 |
| 05:00 | 10 | 17 | 6 | 5 | 9 | 2 | 8 | 9 | 2 | 3 | 3 | 0 |
| 06:00 | 8 | 23 | 8 | 3 | 5 | 2 | 11 | 14 | 2 | 2 | 2 | 1 |
| 07:00 | 16 | 38 | 13 | 9 | 16 | 4 | 16 | 19 | 4 | 8 | 11 | 3 |
| 08:00 | 19 | 41 | 14 | 12 | 19 | 4 | 18 | 22 | 4 | 11 | 13 | 2 |
| 09:00 | 22 | 43 | 13 | 13 | 20 | 4 | 19 | 21 | 3 | 10 | 12 | 2 |
| 10:00 | 24 | 42 | 13 | 13 | 19 | 5 | 19 | 23 | 6 | 10 | 14 | 3 |
| 11:00 | 24 | 38 | 12 | 14 | 21 | 5 | 17 | 19 | 4 | 9 | 13 | 4 |
| 12:00 | 25 | 39 | 12 | 13 | 20 | 5 | 16 | 20 | 5 | 8 | 10 | 3 |
| 13:00 | 24 | 35 | 11 | 14 | 19 | 5 | 14 | 20 | 5 | 7 | 9 | 3 |
| 14:00 | 24 | 37 | 12 | 16 | 22 | 5 | 14 | 19 | 4 | 7 | 9 | 3 |
| 15:00 | 24 | 41 | 13 | 16 | 21 | 4 | 14 | 17 | 3 | 7 | 9 | 2 |
| 16:00 | 24 | 41 | 13 | 16 | 21 | 4 | 13 | 17 | 4 | 6 | 9 | 4 |
| 17:00 | 22 | 40 | 14 | 16 | 19 | 3 | 16 | 22 | 5 | 6 | 10 | 5 |
| 18:00 | 20 | 38 | 12 | 16 | 19 | 5 | 16 | 19 | 5 | 7 | 14 | 7 |
| 19:00 | 19 | 37 | 13 | 13 | 20 | 5 | 16 | 22 | 6 | 7 | 13 | 6 |
| 20:00 | 17 | 37 | 13 | 10 | 17 | 6 | 13 | 16 | 4 | 4 | 7 | 3 |
| 21:00 | 7 | 18 | 7 | 1 | 2 | 1 | 7 | 8 | 1 | 1 | 2 | 1 |
| 22:00 | 2 | 4 | 1 | 2 | 3 | 1 | 10 | 12 | 3 | 1 | 2 | 1 |
| 23:00 | 4 | 9 | 3 | 3 | 5 | 2 | 8 | 10 | 2 | 1 | 2 | 1 |